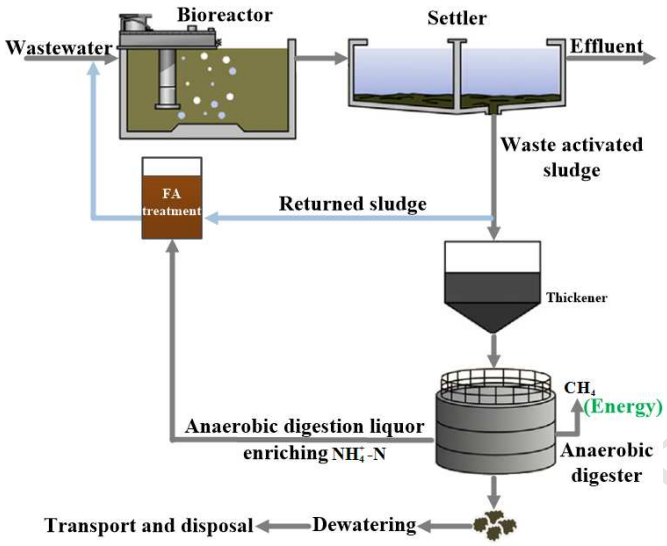

Free Ammonia-Based Sludge Treatment Reduces Sludge Production in the Wastewater Treatment Process

Guojing Yang,[a,b,c] Qiuxiang Xu,[a,b] Dongbo Wang,[a,b,*] Li Tang,[c] Jingfen Xia,[c] Qilin Wang,[d] Guangming Zeng,[a,b] Qi Yang,[a,b,*] Xiaoming Li,[a,b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, P.R. China

[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P.R. China

[c] College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, P.R. China

[d] Griffith School of Engineering & Centre for Clean Environment and Energy, Griffith University, QLD, Australia

Corresponding author

Email: w.dongbo@yahoo.com (Dongbo Wang); yangqi@hnu.edu.cn (Qi Yang)

Tel: 86-731-88823967

Fax: 86-731-88822829

Abstract: Excessive sludge production is one of the major challenges for biological wastewater treatment plants. This paper reports a new strategy to enhance sludge reduction from the wastewater treatment process. In this strategy, 1/5 of the sludge is withdrawn from the mainstream reactor into a side-stream unit for sludge treatment with 16 mg/L free ammonia (FA) for 24-40 h. The FA-treated sludge mixture is then returned to the mainstream reactor. To demonstrate this concept, two reactors treating synthetic domestic wastewater were operated, with one serving as the experimental reactor and the other as the control. Experimental results showed that the experimental reactor exhibited 20% lower in sludge production than the control. FA treatment effectively disintegrated a portion of extracellular or intracellular substances of sludge cells in the FA treatment unit and lowered the observed sludge yields in the mainstream reactor, which were the main reasons for the sludge reduction. Although FA treatment decreased the activities of nitrifiers, denitrifiers, and polyphosphate accumulating organisms in the FA treatment unit, this strategy did not negatively affect the reactor performance and sludge properties of the experimental reactor such as sludge settleability, organic removal, nitrogen removal and phosphorus removal. Further investigation showed that the organics released from the FA treatment process could be used by PAOs and denitrifiers for carbon sources.

Keywords: wastewater treatment plants, sludge reduction, biological nutrient removal, free ammonia

1. Introduction

Wastewater treatment plants (WWTPs) are extensively used for biological wastewater treatment to protect natural water bodies from pollution (Chen et al., 2016; Li et al., 2015; Wang et al., 2012a; 2012b; 2017a). Despite their effective in environmental protection, they are often upset by excessive sludge production (Li et al., 2016; Hu et al., 2018; Wang et al., 2017b; 2017c; 2018a). For instance, it was reported that ~34 million metric tons of sludge (~20% of solids) were produced in China in 2015 (Feng et al., 2015; Xu et al., 2017). The treatment and disposal of excessive sludge is costly, accounting for up to 60% of the total operational cost of a WWTP (Appels et al., 2008; Wang et al., 2013a; Zhao et al., 2017; Xu et al., 2018). To enhance sludge reduction, several sludge reduction technologies such as anaerobic fermentation and anaerobic digestion were successfully developed and extensively implemented in the sludge treatment line in the past decades (Li et al., 2011; Appels et al., 2008; Rittmann et al., 2008; Wang et al., 2015a; 2015b).

Reducing sludge production in the wastewater treatment process (i.e., sludge return line) also attracts much attention, because this method allows sludge reduction in the first place (i.e., source reduction) and decreases the costs of subsequent sludge management (Mahmood and Elliott 2006; Wang et al., 2013b). Besides, sludge contains high levels of organic matters, thus the organics released from sludge reduction in the sludge return line are returned and reused in microbial metabolisms in the wastewater treatment process.

Several sludge treatment methods such as thermal, mechanical, and chemical treatments have been tested and implemented in the sludge return line to disrupt the extracellular polymeric substances and cell envelops (Saby et al., 2002; Dytczak et al., 2007; Wang et al., 2013b). Among them, free nitrous acid (FNA) based technology seems to be promising, as free nitrous acid is a waste-generated, renewable chemical that can be produced in situ in WWTPs by side-stream nitritation of the anaerobic digestion liquor (Law et al., 2015; Wang et al., 2016a; 2016b; 2017d). Wang et al. (2013b) treated 50% of the excess sludge with 2.0 mg/L FNA for 24-42 h, achieving a 28% reduction in sludge production. Compared with untreated sludge, the internal organics, denitrification efficiency, and sludge reduction were demonstrated to be increased by 50%, 76%, and 88%, respectively, in a simultaneous fermentation and denitrification reactor fed with sludge pretreated by FNA (2.04 mg/L) for 24 h (Ma et al., 2015). Despite its effective, this free nitrous acid based technology requires a side-stream nitritation reactor implemented in WWTPs to produce free nitrous acid. Unfortunately, the side-stream nitritation reactor is generally non-existed in most of the current WWTPs.

Free ammonia (FA), the unionized form of ammonium (i.e., NH_3), can be obtained up to ~500 mg NH_3-N/L directly from anaerobic digester effluent, which usually contains 1.0-2.0 g NH_4^+-N/L at a pH of 7.5-8.6 and 33 ˚C (Cervantes. 2009; Fux et al., 2006). Recently, Wei et al. (2017a) found that the sludge biodegradability was enhanced after FA treatment at 420-680 mg NH_3-N/L for 24 h. Based on the findings, it is assumed that sludge reduction might be also achieved using sludge treatment by FA through incorporating a FA treatment unit in the sludge return line to treat part of the return sludge. Unlike free nitrous acid based technology, this FA method does not require the side-stream nitritation reactor, which thereby has significant benefits in real-word applications. To date, however, this hypothesis has never been tested.

The aim of this work is to evaluate whether this FA-based technology can reduce sludge production and to assess whether this strategy affect the performance of wastewater treatment. Two bench scale anaerobic-low dissolved oxygen (DO) sequencing batch reactors receiving synthetic domestic wastewater were operated to demonstrate the feasibilities. One was carried out as the control, and the other included a side-stream FA treatment unit was performed as the experimental reactor. When the experimental reactor achieved in stable operation, FA treatment was implemented through treating 1/5 of the sludge with 16 mg/L FA for 24-40 h. After steady-state operation, sludge reduction between the two reactors was first assessed and compared. Then, details of how FA-based sludge treatment enhances sludge reduction were explored. Finally, the potential impact of FA treatment on the performance of wastewater treatment (e.g., sludge settleability, organic carbon removal and nutrient removal) was also assessed.

2. Materials and methods

2.1. The composition of synthetic wastewater

The synthetic wastewater was used in this study. The synthetic wastewater, unless otherwise described, contained 320 mg CH_3COONa/L, 136 mg NH_4Cl/L, and 22 mg KH_2PO_4/L yielding an influent chemical oxygen demand (COD), NH_4^+-N, and PO_4^{3-}-P of approximately 250, 35, and 5 mg/L, respectively. These values are close to those measured in the real wastewaters in Central South, China. The concentrations of other nutrients in the synthetic wastewater were prepared as below (per liter): 0.01 g $MgSO_4 \cdot 7H_2O$, 0.005 g $CaCl_2$, and 0.5mL of a trace metal solution. The trace metal solution was detailed in our previous publication (Wang et al., 2008).

2.2. The operations of the control and experimental reactors

Two replicate sequencing batch reactors with a working volume of 10 L each were operated and maintained at 25 ± 1 ºC in a temperature controlled room. One reactor was set as the control while the other was selected as the experimental reactor to examine sludge reduction and nutrient removal by introducing an FA treatment unit (Figure 1). Both the reactors were carried out with three cycles daily. Each 8 h cycle of the control reactor (or the experimental reactor) consisted of approximately 120 min anaerobic and 180 min low DO (0.2-0.5 mg/L) periods, followed by 55 min settling, 5 min decanting, and 120 min idle periods. Waste activated sludge, which was withdrawn from the secondary sedimentation tank of a municipal wastewater treatment plant in Changsha, China, was used as the inocula for both two systems.

In the decanting period, 6 L of the supernatant was discharged from both reactors and replaced with 6 L of the synthetic wastewater in the initial 10 min of the anaerobic period. In the low DO period, air was intermittently supplied into the two reactors through using on/off control systems with online DO detectors to maintain the DO level between 0.2 and 0.5 mg/L. The reactors were constantly mixed with magnetic stirrers except for the settling, decanting, and idle periods. The hydraulic retention time (HRT) and sludge retention time (SRT) in both reactors were the same and maintained at approximately 13.3 h and 20 d, respectively. The two reactors were operated for about 5 months, which were divided into two phases:

Phase I (Baseline phase: Day 0-50): In Phase I, FA treatment was not implemented in the experimental reactor. The two reactors were operated the same regime as described above for about 50 d to achieve steady state. SRT in both reactors were maintained at 20 d.

Phase II (Experimental phase: Day 51-150): The control reactor in Phase II was operated the same as in Phase I. Sludge treatment by FA was implemented in the experimental reactor. Approximately 2 L sludge mixture was daily withdrawn from the experimental reactor at the end of the aerobic period and thickened to 400 ml by centrifugation before being transferred to an FA treatment unit. The pH and NH_4^+-N in the FA treatment unit were controlled at 8.5 and 80 mg/L. This gives a calculated FA concentration of 16 mg N/L (T= 25 °C) according to the formula $\{1.214 \times [NH_4^+\text{-N}] \times 10^{pH}\}/\{\exp[6344/(273 + T)] + 10^{pH}\}$ (Anthonisen et al., 1976). After 24 h treatment, the FA-treated sludge was transferred to a sludge storage unit on a daily basis and recirculated to the experimental reactor evenly three times per day in the feeding period. This actually led to various exposure time of sludge mixture to FA (i.e., 24, 32, and 40 h) for the sludge returned to the experimental reactor in the three consecutive cycles.

2.3. Batch tests to examine whether the organics released in the FA treatment unit can be used by PAOs and denitrifiers

To examine whether the organics released in the FA treatment unit can be used by PAOs and denitrifiers, two batch tests (namely, PAOs-Test and Denitrifiers-Test) were operated.

PAOs-Test: One batch reactor was operated with a working volume of 500 mL. One 200 mL mixed liquor was taken from the experimental reactor at the end of the low DO period after stable operation in Phase II, centrifuged, washed and re-suspended in tap water with a final volume of 200 mL before being moved to the reactor. Then, 100 mL of FA-treated sludge mixture was centrifuged (5000 rpm) for 5 min before its supernatant being decanting into the reactor. No additional carbon source was added in batch reactor and pH in the reactor was maintained ~8.0 manually through dosing 0.5 M HCl or 0.5 M $NaHCO_3$. The reactor was operated under anaerobic condition for 90 min through flushing N_2 into the sludge mixture. Samples were taken at 30 min intervals for COD, PO_4^{3-}-P, polyhydroxyalkanoates (PHA), and glycogen analysis.

Denitrifiers-Test: One batch reactor (working volume of 500 mL) was operated and also received the same amounts of biomass withdrawn from the experimental reactor and supernatant of FA-treated sludge mixture, as described above. Afterwards, NO_3^--N stock solution was added into the batch reactor, resulting in an initial NO_3^--N concentration of 35 mg NO_3^--N/L. The reactor was carried out under anoxic condition for 90 min. All other operations were the same as depicted in PAOs-Test. Samples were taken at 15 min intervals for COD, NO_3^--N, and NO_2^--N analysis.

2.4. Determination of the activities of nitrifiers, denitrifiers, and PAOs

The activities of nitrifiers were expressed by the maximum activities of ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). For the measurement of the activities of nitrifiers, the following batch test was carried out. 200 mL sludge mixed liquor was taken from either the experimental reactor or FA treatment unit, centrifuged (5000 rpm for 5 min), and washed three times with tap water to get rid of the residual NH_4^+-N, NO_3^--N and NO_2^--N. Then, the biomass was re-suspended in tap water with a final volume of 200 mL before being evenly divided into two batch reactors. After that, the two reactors received either NH_4^+-N or NO_2^--N stock solution (both at 5.0 g N/L), resulting in an NH_4^+-N (or NO_2^--N) concentration of 35 mg NH_4^+-N/L (or 35 mg NO_2^--N/L). During the whole experiment process, air was adequately supplied to ensure that DO was not limiting (DO > 5 mg/L). pH in the two reactor were

maintained at ~7.5 by addition of 0.5 M HCl or 0.5 M $NaHCO_3$ solution. The test was lasted for 60 min. Samples were taken at 15 min intervals for the analyses of NH_4^+-N and NO_3^--N. The MLVSS concentration in both reactors was measured when the experiment was finished. The activities of AOB and NOB were respectively determined by the biomass-specific NH_4^+-N consumption and NO_3^--N production rates, which were evaluated by dividing the corresponding volumetric rates by the MLVSS concentration.

For the assessment of the activities of denitrifiers, the following batch experiment was performed. A mixed liquor sample (100 mL) was taken out from either the experimental reactor or FA treatment unit, centrifuged, washed and re-suspended in tap water with a final volume of 100 mL before being transferred in the batch reactor. N_2 was flushed through the sludge to get rid of any residual oxygen. CH_3COONa and NO_3^--N stock solution were added into the reactor, resulting in initial COD and NO_3^--N concentrations of 500 mg COD/L and 100 mg NO_3^--N /L, respectively. pH in the reactor was controlled at ~7.5 by dosing 0.5 M HCl or 0.5 M $NaHCO_3$ solution. The tests lasted for 60 min. Mixed liquor samples were taken regularly (15 min intervals) for NO_3^--N analysis. The MLVSS concentrations were measured at the end of the test. The activities of denitrifiers were determined as the biomass-specific NO_3^--N reduction rate, which was calculated by dividing the volumetric NO_3^--N reduction rate by the MLVSS concentration.

For the assessment of the activity of PAOs, a mixture (100 mL) was taken out from either the experimental reactor or FA treatment unit, centrifuged, washed and re-suspended in tap water with a final volume of 100 mL before being transferred in one batch reactor. Then, the COD concentration in the liquid of the reactor was controlled at 500 mg/L (prepared by CH_3COONa). pH in the reactor was maintained ~8.0 manually through dosing 0.5 M HCl or 0.5 M $NaHCO_3$. The reactor was operated as the anaerobic (2 h)-low DO (0.2~0.5 mg/L, 3h) regime. Samples were taken at 30 min intervals for PO_4^{3-}-P analysis. The activity of PAOs was determined by the biomass-specific PO_4^{3-}-P release or uptake rate.

2.5. Sampling and analytical methods

The mixed liquor volatile solid (MLSS) and MLVSS concentrations in the two reactors were measured twice a week in Phase I and once a week in Phases II. Sludge reduction was determined by comparing the quantity of sludge produced from the control and experimental reactors. The effluent NH_4^+-N, NO_3^--N, NO_2^--N, and COD concentrations were measured twice a week. SVI (sludge volume index, twice a week) and particle size distribution in sludge (once a week) were also determined.

All samples were taken using a pipette and immediately filtered through a Whatman GF/C glass microfiber filter (0.22 μm pore size) for the measurements of NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P and COD in accord with standard methods (APHA, 1998). The MLSS and MLVSS concentrations as well as SVI were determined according to the standard methods (APHA, 1998). Particle size distribution analysis was performed using a Malvern Mastersizer 2000 instrument (Malvern Instruments, Worcestershire, UK) with a detection range of 0.01 ~ 3500 μm. The observed sludge yield (Y_{obs}: mg MLSS/mg COD) was calculate by sludge production rate to the COD removed in the reactor (Metcalf and Eddy, 2003). The determination of PHA (calculated as the sum of poly-3-hydroxybutyrate, poly-3-hydroxyvalerate, and poly-3-hydroxy-2-methylvalerate) and glycogen was conducted the same as described prior studies (Wang et al., 2009). Excitation emission matrix (EEM) fluorescence spectroscopy ((Fluoromax-4 Spectrofluorometer, HORIBA Scientific, France) with a 450 W Xe arc lamp) was utilized to characterize the changes of FA-treated sludge liquid according to the method reported previously (Luo et al., 2013). Fluorescence in situ hybridization was used to quantify the abundances of PAOs, AOB, and NOB in the two reactors, and the procedures were detailed prior studies (Wang et al., 2012b; 2016b).

2.6. Statistical Analysis

An analysis of variance was used to evaluate the significance of results, and $p < 0.05$ ($p > 0.05$) was considered to be statistically significant (insignificant).

3. Results and discussion

3.1. Comparison of sludge production between the two reactors

Fig. 2 shows that the concentrations of MLSS and MLVSS in the two reactors during the long-term operation. It can be seen that both the MLSS and MLVSS concentrations in the control reactor remained stable over the whole operation period. In Phase I (i.e., before implementing FA treatment), the experimental reactor had MLSS and MLVSS concentrations similar to those in the control reactor. When FA treatment was implemented, the MLSS and MLVSS concentrations decreased gradually during the initial two weeks and then remained stable in the remainder period.

To further assess feasibility of FA-based sludge treatment achieves sludge reduction, Table 1 summaries the concentrations of MLSS and MLVSS as well as sludge production rates in the reactors in stable operation

196 before and after implementing FA treatment. In steady-state operation of Phase I, MLSS and MLVSS in the
197 two reactors were similar and maintained at ~2200 mg/L and ~1900 mg/L, respectively. When FA treatment
198 was implemented in the experimental reactor, MLSS and MLVSS concentrations reduced to ~1800 mg/L and
199 ~1500 mg/L, respectively. Sludge production rate in this reactor decreased from ~1130 to ~920 mg MLSS/d.
200 According to our calculation, a sludge reduction of approximately 20% was achieved in the experimental
201 reactor in stable operation of Phase II using FA treatment.

202 *3.2. Details of how FA treatment causes sludge reduction*

203 In order to reveal the details of how FA treatment achieves sludge reduction, we first figured out the fact
204 of what happens in FA treatment unit. Sludge cells mainly consist of extracellular polymeric substances and
205 intracellular substances, and their releases could be indicated by EEM fluorescence spectroscopy. Previous
206 studies confirmed that protein comprises large amount of aromatic structures, which have fluorescence
207 characteristics. In this work, EEM was used to characterize the changes of sludge liquid before (Fig. 3a) and
208 after FA treatment (Fig. 3b). The Peak A and Peak B, which are respectively located at the Ex/Em of
209 275-280/ 335-355 and 220-225/335-345 nm in EEM spectra and related to tryptophan protein-like substances
210 (peak A) and aromatic proteins (peak B) (Sheng and Yu, 2006; Luo et al., 2013), were observed in both
211 samples. Compared with the untreated sludge, FA-treated sample had stronger the fluorescence intensities of
212 the peaks A-B in EEM spectra, indicating that more protein was present in the sludge supernatant liquor after
213 FA treatment. The results suggested that FA treatment led to either extracellular polymeric substances or
214 intracellular substances released from solid phase to liquid phase (Wang et al., 2018b; Wei et al., 2017a).

215 Fig. 3c shows the profiles of cumulative floc size distribution of sludge flocs before and after FA
216 treatment. The sludge with 24h FA treatment performed a shift in particle size distribution towards smaller
217 sizes with the peak at 79-104 um than sludge without FA treatment, which had the most volume density
218 between 181 and 208 um, suggesting that FA treatment decreased the floc size of sludge. All these results
219 indicated that FA treatment caused the disruption of sludge flocs. It was reported that FA could break down
220 the recalcitrant structure of sludge flocs and led to a rapid increase of soluble substrates (Hejnfelt and
221 Angelidaki, 2009).

222 Table 2 further exhibits the variations of soluble COD, NH_4^+-N, and activities of nitrifiers, denitrifiers,
223 and PAOs in FA treatment unit before and after FA treatment at steady-state in Phase II. It was found that FA

224 treatment increased the concentrations of soluble COD and NH_4^+-N. After 24 h FA treatment, 729 ± 9 mg/L
225 of soluble COD and 90.8 ± 3.6 mg/L of NH_4^+-N were measured, respectively. This confirmed again that FA
226 treatment could enhance the disruption of sludge flocs. Previous studies also verified that the extracellular
227 materials of sludge cells were disrupted into debris by FA treatment (Wei et al., 2017a; 2017b), which was in
228 accord with the findings obtained in this work. The direct disruption of sludge flocs was one reason for the
229 enhanced sludge reduction achieved in the experimental reactor.

230 Also from Table 2, it can be seen that the activities of AOB, NOB, denitrifiers, and PAOs were all
231 inhibited by FA treatment. The activities of AOB and NOB decreased from 9.8 ± 0.6 and 8.1 ± 0.7 mg N/g
232 VSS•h to 7.7 ± 0.7 and 4.3 ± 0.5 mg N/g VSS•h after 24 h FA treatment, respectively, suggesting that FA
233 treatment resulted in a 21% decrease in AOB activity and a 47% decrease in NOB activity. Similar to
234 nitrifiers, the activity of denitrifiers was inhibited by 36% after 24 h FA treatment (the denitrification rate
235 decreased from 95.6 ± 3.6 to 61.2 ± 3.1 mg NO- 3-N/g VSS h). However, the activity of PAOs was observed
236 to be inhibited severely (>90%). After 24 h treatment by FA, anaerobic phosphate release rate decreased
237 from 20.9 ± 1.4 to 1.7 ± 0.4 mg P/g VSS•h while aerobic phosphate uptake rate decreased from 14.2 ± 0.9 to
238 0.8 ± 0.3 mg P/g VSS•h. It is well-known that the enzymes of these bacteria are cell membrane-bound
239 enzymes which are protected by extracellular polymeric substances. When sludge cells especially
240 extracellular polymeric substances are disrupted by the biocidal effect of FA, these membrane-bound enzymes
241 are easier to be attacked by FA. Previous investigation showed that FA could diffuse through the cell
242 membrane, shuttle protons between the two sides without energy consumption, and caused cell inactivation by
243 disrupting the proton and potassium balance inside the cell (Hejnfelt and Angelidaki, 2009). Possibly due to
244 this inactivation, a lower Y_{obs} (observed sludge yields) was achieved in the experimental reactor at steady
245 Phase II, as compared with that in the control (0.21 ± 0.01 vs 0.25 ± 0.01 mg MLSS/mg COD), it can be
246 understood why lower sludge production was achieved in the experimental reactor.

247 *3.3. Effect of FA treatment on sludge properties and reactor performance*

248 In Phase I (i.e., before implementing FA treatment), similar reactor performance was observed in the
249 control and experimental reactors (i.e., effluent COD, NH_4^+-N, NO_3^--N, NO_2^--N, and PO_4^{3-}-P concentrations) and
250 sludge properties (i.e., SVI, particle size, and activities and abundances of AOB, NOB, and PAOs) (please see
251 Table S1 for details). For instance, 0.5 and 0.4 mg/L of PO_4^{3-}-P, 0.2 and 0.3 mg/L of NH_4^+-N, 0.1 and 0.2

mg/L of NO_2^--N, and 2.0 and 2.3 mg/L of NO_3^--N were detected in the control and experimental reactors, respectively, suggesting that more than 90% of phosphate removal and 92% of nitrogen removal were achieved in both reactors.

To assess whether FA treatment affects the reactor performance and sludge properties, we compared the parameters above of the two reactors at steady-state Phase II, and the results were summarized in Table 3. It was observed that particle size (d50) in the experimental reactor was ~10% lower than that in the control reactor (Table 3 and Fig. 4). As discussed in Section 3.2, FA treatment could disintegrate extracellular materials and/or the bacterial cells. This is possibly to result in the generation of smaller particles and then accumulated in the experimental reactor, which might be the reason for the relatively smaller particle sizes in the experimental reactor. Although FA treatment decreased particle size of sludge cells, sludge settleability was not affected by this treatment, which was reflected by SVI. It was found that 134 ± 5 and 142 ± 7 mL/g of SVI were respectively measured in the control and experimental reactors, and this difference was found to be statistically insignificant ($p > 0.05$).

The activities and abundances of AOB and NOB in the two reactors were detected to evaluate if FA treatment affects nitrifiers. It can be seen that there was no significant change ($p > 0.05$) in terms of the activities of AOB and NOB between the control and experimental reactor at steady Phase II, suggesting that the activities of nitrifiers were not affected when FA treatment was incorporated. In addition, the populations of AOB and NOB in the control reactor were also similar to those in the experimental reactor ($p > 0.05$). The abundances of AOB and NOB were respectively 6.5 ± 0.8% and 3.3 ± 0.4% in the control reactor, while the corresponding data were 7.4 ± 1.1% and 3.1 ± 0.3%, respectively. Moreover, the activity of denitrifiers was also similar in the control and experimental reactors (97.6 ± 5.9 vs 93.9 ± 4.7 mg N/g VSS•h). These results demonstrated that both nitrification and denitrification in the mainstream reactor was unaffected by FA treatment. As a result, the two reactors had almost the same NH_4^+-N, NO_3^--N, and NO_2^--N in the effluent (Table 3).

As shown in Table 2, the activity of PAOs in the FA treatment unit was severely inhibited by FA for 24 h (>90%). However, this inhibition was largely mitigated in the mainstream experimental reactor. In stable operation, 15.7 ± 0.7 mg P/g VSS•h of anaerobic phosphate release rate and 10.6 ± 0.4 mg P/g VSS•h of aerobic phosphate uptake rate were respectively measured in the experimental reactor, which were 77.3% and

75.7% of those determined in the control reactor. The results indicated that the decreased activity of PAOs in the FA treatment unit was probably due to inhibition rather than inactivation, and the activity of PAOs could be recovered after several days of recovery time. In the literature, Wang et al. also found that the activities of AOB and NOB could be recovered to some extents after 4 d when they were treated by free nitrous acid (Wang et al., 2016b). Further investigation verified that the abundance of PAOs did not decrease largely, which was consistent with the data of anaerobic phosphate release and aerobic phosphate uptake rates. Therefore, an excellent phosphate removal was also obtained in the experimental reactor at steady state operation, which was comparable with that in the control reactor (0.6 ± 0.1 mg/L vs 0.4 ± 0.1 mg/L of phosphate in effluent).

3.4. Could the organics released in the FA treatment unit be used by PAOs and denitrifiers

FA treatment caused a large amount of organics released from sludge cells into liquid phase. After 24 h treatment, 729 ± 28 mg/L soluble COD was measured in the FA treatment unit (Table 2). This soluble COD increased influent organic loading by ~6% in the experimental reactor. However, no significant increase of effluent COD was measured in the experimental reactor, as compared with that in the control reactor (Table 3, $p > 0.05$). This suggested that the organics released from sludge flocs could be metabolized in the experimental reactor. It is known that many biodegradable organics could be degraded under aerobic conditions by ordinary heterotrophic bacteria through the tricarboxylic acid cycle, however, the question as to whether the organics released from sludge flocs could be used by PAOs and denitrifiers for supplementary carbon sources remained unknown.

To assess this feasibility, batch tests were operated using the organics released as the sole carbon source, and the results were outlined in Table 4. It can be seen that soluble COD decrease coupled with anaerobic phosphate release and PHA accumulation occurred concurrently in PAOs-test. The behavior observed was similar to the typical phenotype of PAOs. It should be emphasized that 163 ± 8 mg/L of soluble COD was still determined after 90 min anaerobic period. Moreover, the levels of phosphate release and PHA accumulation were lower than those reported previously (Wang et al., 2012a; 2013a; Yi et al., 2017). The results suggested that not all but only a part of organics released could be metabolized by PAOs. The released organics included 52.3% protein, 19.8% carbohydrate, 5.4% volatile fatty acids, and 22.5% other organic compounds (Table 2). Previous investigations demonstrated that some of them such as acetate,

propionate, glucose, and alcohol could be used by PAOs, and no information was available to the feasibility that protein could be utilized by PAOs before its degradation to small organics (Oehmen et al., 2007; Yang et al., 2018; Wang et al., 2008; 2012a; 2013a).

In Denitrifiers-Test, both soluble COD and NO_3^--N concentrations decreased with the reaction time. At 90 min anoxic time, 135 ± 6 mg/L soluble COD was measured while nitrate was at non-detectable level. The results clearly verified that the organics released could be used as carbon sources for denitrification. By comparing the endogenous denitrification rates in the control and experimental reactors, Wang et al. also demonstrated the availability of free nitrous acid-treated sludge as a carbon source for denitrification (Wang et al., 2013b).

3.5. Implications

This paper reports for the first time that FA-based sludge treatment is effective in sludge reduction from the wastewater treatment process. This was experimentally demonstrated by comparing MLSS concentration and sludge production rates between the two reactors with and without FA treatment. The recirculation of 2 L of the sludge that was treated using FA at 16 mg N/L for 24-40 h to the experimental reactor, 20% lower sludge production was achieved, as comparing with the control reactor without FA treatment. Meanwhile, the reactor performances and sludge properties were not affected by this novel FA-based sludge reduction strategy, suggesting that FA has good potential to be developed into a practical technology in field situations.

In the past years, several sludge treatment methods such as thermal, mechanical, and chemical treatments have been tested and implemented in the sludge return line to enhance sludge reduction (Saby et al., 2002; Dytczak et al., 2007; Wang et al., 2013b). Despite these effective, these methods require high input of either chemicals or energy, which diminish their values in real-world applications. In comparison, however, FA is a waste-generated, renewable chemical that can be produced in situ in WWTPs as a byproduct of sludge treatment (i.e., FA can be attained directly from anaerobic digester/fermenter effluent), suggesting that the FA-based sludge treatment method does not have these drawbacks (Wang et al., 2017e). The findings achieved in this work, therefore, may guide engineers to develop more economic and practical strategies for sludge reduction from the sludge return line.

Fig. 5b presents a “closed-loop” concept in a WWTP for the enhanced sludge reduction from the sludge return line based on the proposed FA-based sludge treatment technology. Apart from returning a part of

sludge, the excess sludge withdrawn from the settler was generally digested anaerobically to reduce the amount of sludge, kill the pathogenic microorganisms, and produce energy biogas methane (Appels et al., 2008; Wang et al., 2015b). The digestion liquid, which usually contains 1.0-2.0 g NH_4^+-N/L at a pH of 7.5-8.6 and 33 ˚C (Cervantes. 2009; Fux et al., 2006), requires to be recirculated to the head of the WWTP to be cleaned before its final discharge (Fig. 4a). By setting up a FA treatment unit in the sludge return line and introducing the digestion liquid into the FA treatment unit instead of the head of WWTP, this FA-based sludge reduction strategy can be readily implemented (Fig. 5b).

Based on the experimental data obtained and key operating parameters used in this work, an expanded research on a full-scale WWTP with a 100,000 population equivalent (PE) was used to investigate the potential economic benefit of this FA-based technology (Please see Table S2 for details). It was estimated that compared to an extended aeration WWTP, total volume of the reactor (bioreactor + FA treatment reactor) of a conventional WWTP based on FA treatment would be reduced by 20% with the same sludge production. This is equivalent to capital investment savings of $1,171,200, with an annual $123,500 saving over a 20-year period by assuming an interest rate of 8.5%. Furthermore, requiring a smaller footprint is crucial and beneficial for WWTPs under the condition of limited land availability. In terms of energy consumption, the annual cost of the system with FA treatment (mixing + recirculation) was estimated to be $50,430 lower compared to the extended aeration system (mixing). The FA treatment unit is expected to consist of a very simple vessel and simple mixing devices, which is the opposite of those currently available methods (i.e. thermal, mechanical and chemical) that require professional vessels and equipment to meet high temperature, high pressure and high mechanical forces conditions. Moreover, FA is a renewable, cheap chemical that can be obtained in-situ production in a WWTP. Thus, the cost of FA and FA treatment unit is very low. In summary, WWTP with the FA method would save $172,930/year. The FA strategy for achieving sludge reduction is economically feasible and environmentally friendly.

It should be emphasized that this study mainly aimed to assess the feasibility of whether FA treatment could reduce sludge production, thus technical optimization was not performed. For example, several parameters such as the FA level, treatment time, and sludge portion for treatment were not optimized. In addition, one bench-scale experiment was tested in this work. Thus, the benefit and cost values presented above should be considered indicative only, its technical and economic feasibilities under real-world situations

364 require to be further evaluated under real-world situations.

365 **4. Conclusions**

366 This paper presents a new strategy about sludge reduction in wastewater treatment process using FA
367 treatment. Experimental results showed that when 2 L sludge was treated with 16 mg N/L FA for 24-40 h
368 every day, 20% of sludge reduction was achieved in the experimental reactor. FA treatment caused the
369 release of large amounts of extracellular or intracellular substances in the FA treatment unit and lowered the
370 observed sludge yields in the mainstream reactor, which were the main reason for the sludge reduction.
371 Although FA treatment decreased the activities of function bacteria in the FA treatment unit, no adverse
372 impacts on reactor performance and sludge properties were found in the mainstream reactor. Further
373 investigation showed that the organics released in the FA treatment process could be used by PAOs and
374 denitrifiers.

375 **Acknowledgements**

376 This study was financially supported by the National Natural Science Foundation of China (51508178,
377 51408551, and 51521006) and Planned Science and Technology Project of Zhejiang Province and Ningbo
378 City, China (2015C33012 and 2015A610253).

379 **Supporting Information**

380 This file contains Tables S1-S2.

381 **References**

382 Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., Srinath, E. G., 1976. Inhibition of nitrification by ammonia and
383 nitrous acid. J. Water Pollut. Control Fed. 48, 835-852.

384 APHA (American Public Health Association), 1998. Standard Methods for the Examination of Water and Wastewater,
385 20th ed. Washington, DC, USA.

386 Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of
387 waste-activated sludge. Prog. Energ. Combust. 34, 755-781.

388 Cervantes, F. J. Environmental Technologies to Treat Nitrogen Pollution; IWA Publishing: London, U.K., 2009.

389 Chen, H., Liu, Y., Ni, B., Wang, Q., Wang, D., Zhang, C., Li, X., Zeng, G., 2016. Full-scale evaluation of
390 aerobic/extended-idle regime inducing biological phosphorus removal and its integration with intermittent sand
391 filter to treat domestic sewage discharged from highway rest area. Biochem. Eng. J. 113, 114-122.

392 Dytczak, M.A., Londry, K.L., Siegrist, H., Oleszkiewicz, J.A., 2007. Ozonation reduces sludge production and improves

denitrification. Water Res. 41, 543-550.

Feng, L., Luo, J., Chen, Y., 2015. Dilemma of sewage sludge treatment and disposal in China. Environ. Sci. Technol. 49, 4781-4782.

Fux, C., Velten, S., Carozzi, V., Solley, D., Keller, J. 2006. Efficient and stable nitritation and denitritation of ammonium-rich sludge dewatering liquor using an SBR with continuous loading. Water Res. 40 (14), 2765−2775.

Hejnfelt, A., Angelidaki, I., 2009. Anaerobic digestion of slaughterhouse by-products. Biomass Bioenergy. 33, 1046-1054.

Hu, J., Zhao, J., Wang, D., Li, X., Zhang, D., Xu, Q., Peng, L., Yang, Q., Zeng, G., 2018. Effect of Diclofenac on the Production of Volatile Fatty Acids from Anaerobic Fermentation of Waste Activated Sludge. Bioresour. Technol. 254, 7-15.

Law, Y., Ye, L., Wang, Q., Hu, S., Pijuan, M., Yuan, Z., 2015. Producing free nitrous acid - A green and renewable biocidal agent - From anaerobic digester liquor. Chem. Eng. J. 259, 62-69.

Li, W., Yu, H., Rittmann, B. E., 2015. Reuse water pollutants. Nature, 528, 29-31.

Li, X., Chen, H., Hu, L., Yu, L., Chen, Y., Gu, G., 2011. Pilot-scale waste activated sludge alkaline fermentation, fermentation liquid separation, and application of fermentation liquid to improve biological nutrient removal. Environ. Sci. Technol. 45, 1834-1839.

Li, X., Zhao, J., Wang, D., Yang, Q., Xu, Q., Deng, Y., Yang, W., Zeng, G., 2016. An efficient and green pretreatment to stimulate short-chain fatty acids production from waste activated sludge anaerobic fermentation using free nitrous acid. Chemosphere 144, 160-167.

Luo, K., Yang, Q., Li, X., Chen, H., Liu, X., Yang, G., Zeng, G., 2013. Novel insights into enzymatic-enhanced anaerobic digestion of waste activated sludge by three-dimensional excitation and emission matrix fluorescence spectroscopy. Chemosphere, 91, 579-585.

Ma, B., Peng, Y., Wei, Y., Li, B., Bao, P., Wang, Y., 2015. Free nitrous acid pretreatment of wasted activated sludge to exploit internal carbon source for enhanced denitrification. Bioresour. Technol. 179, 20-25.

Mahmood, T., Elliott, A., 2006. A review of secondary sludge reduction technologies for the pulp and paper industry. Water Res. 40, 2093-2112.

Metcalf and Eddy, 2003. Wastewater Engineering: Treatment and Reuse, fourth ed. McGraw Hill Education, ISBN 0070418780.

Oehmen, A., Lemos, P.C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L.L., Reis, M.A.M., 2007. Advances in enhanced biological phosphorus removal: From micro to macro scale. Water Res 41, 2271–2300.

423 Rittmann, B.E., Lee, H.S., Zhang, H., Alder, J., Banaszak, J.E., Lopez, R., 2008. Full-scale application of focused-pulsed
424 pretreatment for improving biosolids digestion and conversion to methane. Water Sci. Technol. 58, 1895-1901.
425 Saby, S., Djafer, M., Chen, G., 2002. Feasibility of using a chlorination step to reduce excess sludge in activated sludge
426 process. Water Res. 36, 656-666.
427 Sheng, G., Yu, H., 2006. Characterization of extracellular polymeric substances of aerobic and anaerobic sludge using
428 three-dimensional excitation and emission matrix fluorescence spectroscopy. Water Res. 40, 1233-1239.
429 Wang, D., Li, X., Yang, Q., Zeng, G., Liao, D., Zhang, J., 2008. Biological phosphorus removal in sequencing batch
430 reactor with single-stage oxic process. Bioresour. Technol. 99, 5466-5473.
431 Wang, D.B., Li, X.M., Qi, Y., Wei, Z., Liu, Z.Y., Liu, Y.L., Cao, J.B., Yue, X., Shen, T.T., Zeng, G.M., Deng, J.H., 2009.
432 The probable metabolic relation between phosphate uptake and energy storages formations under single-stage oxic
433 condition. Bioresour. Technol. 100, 4005-4011.
434 Wang, D., Li, X., Yang, Q., Zheng, W., Wu, Y., Zeng, T., Zeng, G., 2012a. Improved biological phosphorus removal
435 performance driven by the aerobic/extended-idle regime with propionate as the sole carbon source. Water Res. 46,
436 3868-3878.
437 Wang, D., Yang, G., Li, X., Zheng, W., Wu, Y., Yang, Q., Zeng, G., 2012b. Inducing mechanism of biological phosphorus
438 removal driven by the aerobic/extended-idle regime. Biotechnol. Bioeng. 109, 2798-2807.
439 Wang, D., Zheng, W., Li, X., Yang, Q., Liao, D., Zeng, G., 2013a. Evaluation of the Feasibility of Alcohols Serving as
440 External Carbon Sources for Biological Phosphorus Removal Induced by the Oxic/Extended-Idle Regime.
441 Biotechnol. Bioeng. 110, 827-837.
442 Wang, Q., Ye, L., Jiang, G., Yuan, Z., 2013b. A free nitrous acid (FNA)-based technology for reducing sludge production.
443 Water Res. 47, 3663-3672.
444 Wang, D., Zeng, G., Chen, Y., Li, X. M., 2015a. Effect of polyhydroxyalkanoates on dark fermentative hydrogen
445 production from waste activated sludge. Water Res. 73, 311-322.
446 Wang, D., Zhao, J., Zeng, G., Chen, Y., Bond, P. L., Li, X., 2015b. How does poly (hydroxyalkanoate) affect methane
447 production from the anaerobic digestion of waste-activated sludge? Environ. Sci. Technol. 49, 12253-12262.
448 Wang, D., Wang, Q., Laloo, A. E., Yuan, Z., 2016a. Reducing N_2O emission from a domestic-strength nitrifying culture
449 by free nitrous acid-based sludge treatment. Environ. Sci. Technol. 50, 7425-7433.
450 Wang, D., Wang, Q., Andrew, L., Xu, Y., Bond, P. L., Yuan, Z., 2016b. Achieving stable nitritation for mainstream
451 deammonification by combining free nitrous acid-based sludge treatment and oxygen limitation. Sci. Rep. 2016, 6,
452 25547.

Wang, D., Wang, Y., Liu, Y., Ngo, H. H., Lian, Y., Zhao, J., Chen, F., Yang, Q., Zeng, G., Li, X., 2017a. Is Denitrifying Anaerobic Methane Oxidation-Centered Technologies a Solution for the Sustainable Operation of Wastewater Treatment Plants? Bioresour. Technol. 234, 456-465.

Wang, D., Liu, Y., Ngo, H. H., Zhang, C., Yang, Q., Peng, L., He, D., Zeng, G., Li, X., Ni, B., 2017b. Approach of describing dynamic production of volatile fatty acids from sludge alkaline fermentation. Bioresour. Technol. 238, 343-351.

Wang, Y., Wang, D., Yang, Q., Zeng, G., Li, X., 2017c. Wastewater opportunities for denitrifying anaerobic methane oxidation. Trends Biotechnol. 35, 799-802.

Wang, D., Fu, Q., Xu, Q., Liu, Y., Ngo, H. H., Yang, Q., Zeng, G., Li, X., Ni, B. J. 2017d. Free nitrous acid-based nitrifying sludge treatment in a two-sludge system enhances nutrient removal from low-carbon wastewater. Bioresour. Technol. 244, 920-928.

Wang, Q., Duan, H., Wei, W., Ni, B. J., Laloo, A., Yuan, Z. 2017e. Achieving Stable Mainstream Nitrogen Removal via the Nitrite Pathway by Sludge Treatment Using Free Ammonia. Environ. Sci. Technol. 51(17), 9800-9807.

Wang, D., Liu, X., Zeng, G., Zhao, J., Liu, Y., Wang, Q., Chen, F., Li, X., Yang, Q., 2018a. Understanding the Impact of Cationic Polyacrylamide on Anaerobic Digestion of Waste Activated Sludge. Water Res. 130, 281-290.

Wang, D., Duan, Y., Yang, Q., Liu, Y., Ni, B. J., Wang, Q., Zeng, G., Li, X., Yuan, Z. 2018b. Free ammonia enhances dark fermentative hydrogen production from waste activated sludge. Water Res. 133, 272-281.

Wei, W., Zhou, X., Wang, D., Sun, J., Wang, Q., 2017a. Free ammonia pre-treatment of secondary sludge significantly increases anaerobic methane production. Water Res. 118, 12-19.

Wei, W., Zhou, X., Xie, G.-J., Duan, H., Wang, Q. 2017b. A novel free ammonia based pretreatment technology to enhance anaerobic methane production from primary sludge. Biotechnol. Bioeng., 114: 2245–2252.

Xu, Q., Li, X., Ding, R., Wang, D., Liu, Y., Wang, Q., Zhao, J., Chen, F., Zeng, G., Yang, Q., Li, H., 2017. Understanding and mitigating the toxicity of cadmium to the anaerobic fermentation of waste activated sludge. Water Res. 124, 269-279.

Xu, Q., Liu, X., Zhao, J., Wang, D., Wang, Q., Li, X., Yang, Q., Zeng, G., 2018. Feasibility of enhancing short-chain fatty acids production from sludge anaerobic fermentation at free nitrous acid pretreatment: Role and significance of Tea saponin. Bioresour. Technol.254, 194-202.

Yang, G., Wang, D., Yang, Q., Zhao, J., Liu, Y., Wang, Q., Zeng, G., Li, X., Li, H., 2018. Effect of acetate to glycerol ratio on enhanced biological phosphorus removal. Chemosphere 196, 78-86.

Yi, K., Wang, D., Yang, Q., Li, X., Chen, H., Sun, J., An, H., Wang, L., Deng, Y., Liu, J., Zeng, G., 2017. Effect of

Ciprofloxacin on Biological Nitrogen and Phosphorus Removal from Wastewater. Sci. Total Environ. 2017, 605–606, 368–375.

Zhao, J., Gui, L., Wang, Q., Liu, Y., Wang, D., Ni, B., Li, X., Xu, R., Zeng, G., Yang, Q., 2017. Aged Refuse Enhances Anaerobic Digestion of Waste Activated Sludge. Water Res. 123, 724-733.

Figure legends

Fig. 1. Schematic diagram of the control (A) and experimental systems (B).

Fig. 2. Long-term MLSS and MLVSS concentrations in the control and experimental reactors. Phase I: before FA treatment (baseline phase); Phase II: FA treatment implemented in the experimental reactor (experimental phase).

Fig. 3. EEM profiles of liquid (a: before treatment; b: after treatment) and particle size distribution of sludge (c) before and after FA treatment in the FA treatment unit.

Fig. 4. Particle size distribution of sludge in the control and experimental treatment reactors.

Fig. 5. Traditional wastewater treatment process (a), innovative "closed-loop" concept in wastewater treatment process based on the proposed FA treatment technology to achieve sludge reduction (b).

Table legends

Table 1 Summarization of the results associated with sludge reduction.

Table 2 Changes of soluble COD, NH_4^+-N and activities of nitrifiers, denitrifiers, and PAOs in FA treatment unit.

Table 3 Treatment performance and sludge properties of the control and experimental reactors at steady phase II.

Table 4 Changes of soluble COD, PO_4^{3-}-P, PHA, glycogen in PAOs-Test, and variations of COD, and NO_3^--N in Denitrifiers-Test.

Table 1 Summarization of the results associated with sludge reduction [a]

Parameter	Phase I		Phase II	
	Control reactor	Experimental reactor	Control reactor	Experimental reactor
MLSS (mg/L)	2242 ± 21	2256 ± 25	2253 ± 24	1807 ± 18
MLVSS (mg/L)	1896 ± 17	1907 ± 20	1911 ± 19	1505 ± 15
Sludge production rate (mg MLSS/d)	1123 ± 16	1128 ± 15	1126 ± 18	924 ± 16
Sludge reduction	Not applicable		(2253-1807)/2253 =20%	
Y_{obs} (mg MLSS/mg COD)	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.21 ± 0.01
MLVSS/MLSS (%)	84.6 ± 0.1	84.2 ± 0.1	85.0 ± 0.2	83.3± 0.1

[a] Results are the averages and standard deviations from seven measurements during steady-state in Phase II.

Table 2 Changes of soluble COD, NH_4^+-N and activities of nitrifiers, denitrifiers, and PAOs in FA treatment unit [a]

Treatment time	Soluble COD (mg/L)	NH_4^+-N (mg/L)	AOB activity (mg N/g VSS·h)	NOB activity (mg N/g VSS·h)	P release rate (mg P/g VSS·h)	P uptake rate (mg P/g VSS·h)	Denitrification rate (mg NO_3^--N/g VSS h)
0	15 ± 3	0.2 ± 0.1	9.8 ± 0.6	8.1 ± 0.7	20.9 ± 1.4	14.2 ± 0.9	95.6 ± 3.6
24 h	729 ± 28[b]	90.8 ± 3.6	7.7 ± 0.7	4.3 ± 0.5	1.7 ± 0.4	0.8 ± 0.3	61.2 ± 3.1

[a] Results are the averages and standard deviations from triplicate tests during steady-state in Phase II. The pH in the FA treatment unit was 8.5 during the entire treatment period. [b] The soluble COD included 52.3% protein, 19.8% carbohydrate, 5.4% volatile fatty acids, and 22.5% others.

Table 3 Treatment performance and sludge properties of the control and experimental reactors at steady phase II [a]

Parameter		Control reactor	Experimental reactor
Treatment performance	Effluent COD (mg/L)	13.9 ± 4.2	16.6 ± 3.7
	Effluent PO_4^{3-} (mg/L)	0.4 ± 0.1	0.6 ± 0.1
	Effluent NH_4^+ concentration (mg N/L)	0.2 ± 0.1	0.1 ± 0.1
	Effluent NO_2^- concentration (mg N/L)	0.1 ± 0.1	0.1 ± 0.1
	Effluent NO_3^- concentration (mg N/L)	2.2 ± 0.5	2.5 ± 0.6
Sludge properties	SVI (mL/g)	134 ± 5	142 ± 7
	Particle size (d50) [b] (μm)	206 ± 7	183 ± 10
	AOB abundance (%)	6.5 ± 0.8	7.4 ± 1.1
	AOB activity (mg N/g VSS·h)	7.94 ± 0.12	8.05 ± 0.17
	NOB abundance (%)	3.3 ± 0.4	3.1 ± 0.3
	NOB activity (mg N/g VSS·h)	7.15 ± 0.11	6.52 ± 0.14
	PAO abundance (%)	17.7 ± 3.2	15.6 ± 2.7
	P release rate (mg P/g VSS·h)	20.3 ± 0.5	15.7 ± 0.7
	P uptake rate (mg P/g VSS·h)	14.0 ± 0.3	10.6 ± 0.4
	Denitrifiers' activity (mg N/g VSS·h)	97.6 ± 5.9	93.9 ± 4.7

[a] Results are the averages and standard deviations from triplicate measurements during steady-state in Phase II. [b] d50 denotes the size for which 50% (measured based on volume) of particles in the sample is below this size.

Table 4 Changes of soluble COD, PO_4^{3-}-P, PHA, glycogen in PAOs-Test, and variations of COD, and NO_3^--N in Denitrifiers-Test [a]

Treatment time	PAOs-Test			Denitrifiers-Test	
	Soluble COD (mg/L)	PO_4^{3-}-P (mg/L)	PHA (mmol C/g VSS)	Soluble COD (mg/L)	NO_3^--N (mg/L)
0	242 ± 10	0.1 ± 0.1	0.56 ± 0.12	242 ± 10	35 ± 0.1
90 min	163 ± 8	13.8 ± 0.4	0.84 ± 0.15	135 ± 6	Non-detectable

[a] Results are the averages and standard deviations from triplicate tests.

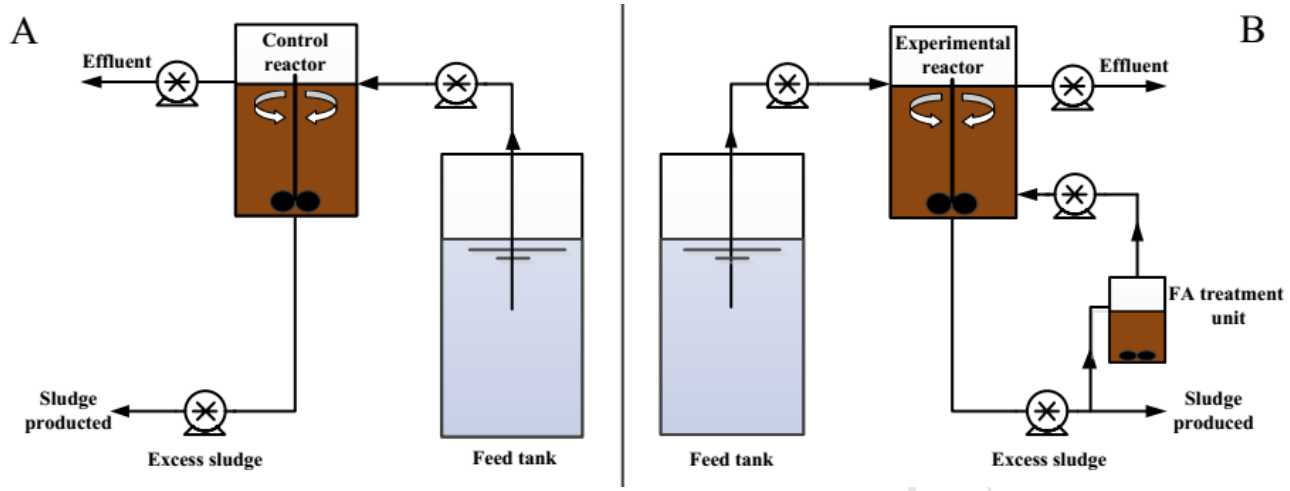

Fig. 1. Schematic diagram of the control (A) and experimental systems (B).

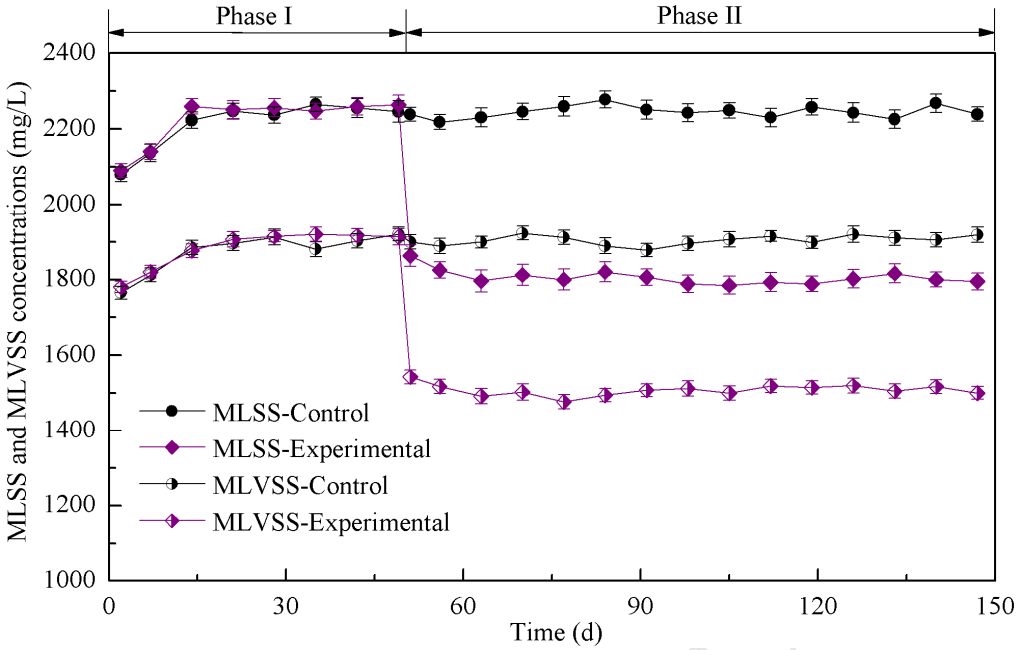

Fig. 2. Long-term MLSS and MLVSS concentrations in the control and experimental reactors. Phase I: before FA treatment (baseline phase); Phase II: FA treatment implemented in the experimental reactor (experimental phase).

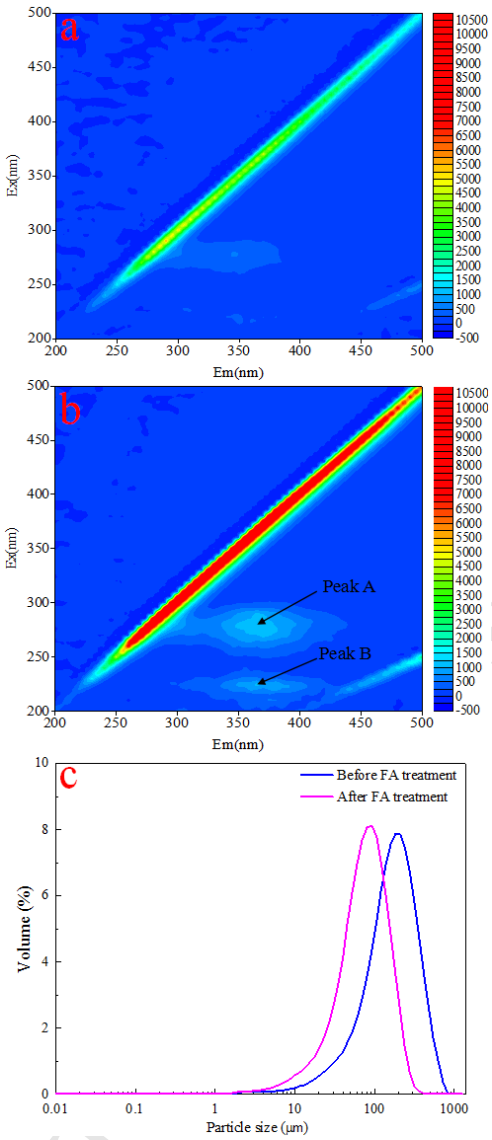

Fig. 3. EEM profiles of liquid (a: before treatment; b: after treatment) and particle size distribution of sludge (c) before and after FA treatment in the FA treatment unit.

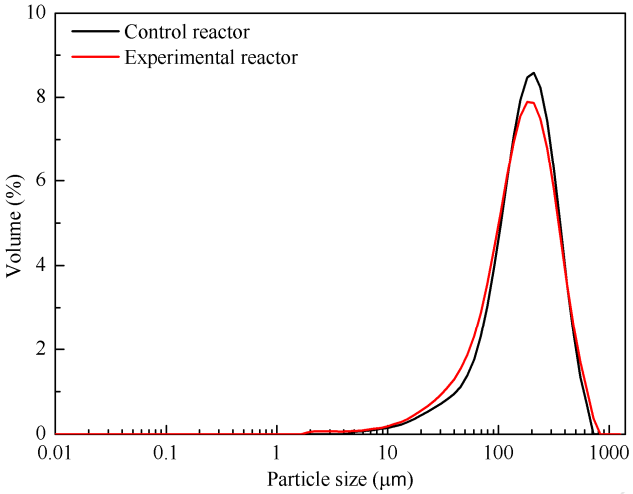

Fig. 4. Particle size distribution of sludge in the control and experimental treatment reactors.

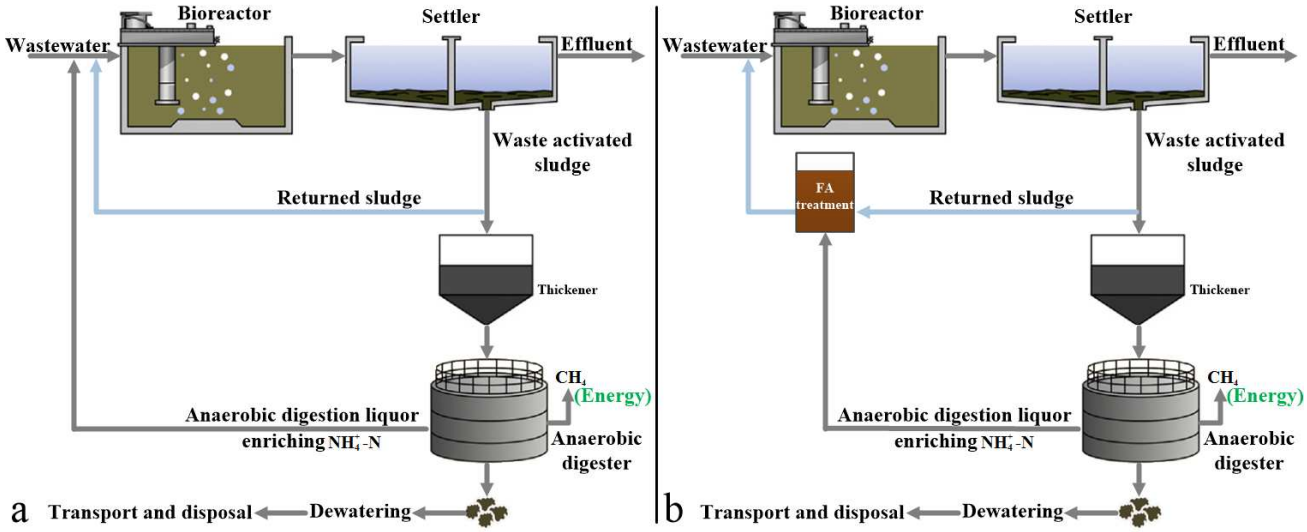

Fig. 5. Traditional wastewater treatment process (a), innovative "closed-loop" concept in wastewater treatment process based on the proposed FA treatment technology to achieve sludge reduction (b).

Highlights:

- A new strategy was developed for sludge reduction from wastewater treatment process.
- The strategy was implemented through treating part of the sludge with FA.
- 16 mg N/L FA caused ~20% of sludge reduction in the experimental reactor.
- Reactor performance and sludge properties were not affected by FA treatment.