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Title	Nitrogen behavior during sludge ozonation: a long-term observation by pilot experiments.
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Citation	Water science and technology (2014), 70(2): 289-296
Issue Date	2014-02
URL	http://hdl.handle.net/2433/200622
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Type	Journal Article
Textversion	author

1 **Nitrogen behavior during sludge ozonation: a long-term observation by pilot experiments**

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11 **ABSTRACT**

12 Sludge ozonation is a promising technology for dealing with the increasing challenge of excess
13 sludge treatment and disposal. However, nitrogen behavior during sludge ozonation and
14 subsequent biological removal remains unclear. To clarify the feasibility and stability of
15 oxidizing organic nitrogen (released during sludge ozonation) in the bioreactor (but not during
16 ozonation), and the best operational conditions for sludge ozonation, the nitrogen behavior was
17 investigated by a long-term observation. The results showed that when inlet ozone concentration
18 increased from 30 to 80 mg O_3/L and ozonation time decreased from 29 to 11 h, less soluble
19 organic nitrogen was oxidized to ammonia (from 66.1% to 18.7% of soluble total nitrogen). This
20 can reduce the operational costs of sludge ozonation. Furthermore, it is feasible to convert
21 organic nitrogen to nitrate by biological processes because full nitrification was restored in three
22 weeks after shock loading of organic nitrogen owing to the change in ozonation conditions. After
23 combining sludge ozonation with the anaerobic/oxic process, the mass balance for nitrogen
24 showed that nitrogen in the excess sludge decreased with increasing sludge reduction rate. The
25 decreased nitrogen in the excess sludge mainly transformed to nitrogen gas by denitrification,
26 whereas nitrogen in the effluent did not increase obviously.

27 **KEYWORDS**

28 Sludge ozonation, nitrogen release, nitrogen removal, sludge reduction

INTRODUCTION

Excess sludge treatment and disposal has been an increasing challenge with the worldwide application of biological wastewater treatment processes. The increasing amounts of excess sludge and the high cost of its treatment and disposal, which accounts for more than 20%, even up to 60%, of the total plant operating costs (Perez-Elvira *et al.* 2006; Ginestet 2007; LeBlanc *et al.* 2008; Foladori *et al.* 2010), have both necessitated the development of excess sludge reduction technologies. In addition, increasingly stringent regulations regarding sludge treatment and disposal, as well as social and environmental concerns, have resulted in a continuous and considerable impetus for further developments.

Most strategies for sludge reduction are based on lysis-cryptic growth, in which biomass grows using released cell content after sludge disintegration. As one of effective ways to break cells, ozonation of sludge can yield the highest degree of sludge disintegration (Chu *et al.* 2009). Ozonation for sludge reduction has been proposed since 1990s (Yasui & Shibata 1994); however, the behavior of inorganic elements during sludge ozonation and their effect on the biological treatment process remain unclear (Sui *et al.* 2011). Particularly, nitrogen release and the composition of the released soluble nitrogen strongly depend on the operational conditions of sludge ozonation because ozone can further oxidize the released organic nitrogen to ammonia and then to nitrogen oxides (Garcia-Orozco *et al.* 2011). It has been reported that inorganic nitrogen accounts for approximately 20% of the released soluble total nitrogen during sludge ozonation for an ozone consumption rate of 100 mg O_3/g SS (Sui *et al.* 2011). To minimize costs and effectively utilize ozone, ozone activity should target sludge particles. However, in the complex matrices of activated sludge, sludge particles, released biodegradable organic compounds (including nitrogen element), and other inorganic compounds will compete to react with ozone (Cesbron *et al.* 2003). Therefore, to save energy and remove nitrogen effectively, it is important to determine the optimum operational conditions for sludge ozonation. The oxidation of organic nitrogen to inorganic nitrogen can occur during sludge ozonation or in a bioreactor. If it occurs during sludge ozonation, the operational costs will increase. If it occurs in the bioreactor, the increased loading rate will probably affect the transformation of nitrogen and its removal rate in anaerobic/oxic (A/O) or anaerobic/anoxic/oxic (A/A/O) processes.

The objectives of the present study are to explore how the oxidation of soluble organic nitrogen can be avoided during ozonation and if the conversion of the released organic nitrogen to ammonia and then nitrate is feasible or brings instability to the subsequent biological steps. We investigated the nitrogen behavior in an advanced sewage treatment process combined with sludge reduction and phosphorus recovery (advanced SRPR process), with a focus on nitrogen release properties during sludge ozonation and subsequent biological removal. Furthermore, we discuss the improved operational mode of sludge ozonation according to the observed behavior of nitrogen compounds during the long-term operation of a pilot-scale experimental system.

MATERIAL AND METHODS

Experimental Apparatus

An advanced SRPR process, which incorporated sludge ozonation and phosphorous crystallization into the A/O process (Saktaywin *et al.* 2005; Saktaywin *et al.* 2006; Nagare *et al.*

70 2008; Tsuno *et al.* 2008), was employed in this study. In this process (Figure 1), settled
71 phosphorus-rich supernatant at the end of the anaerobic stage is taken out of the bioreactor for
72 phosphorus recovery, and the excess sludge is treated with ozone and then returned to the
73 bioreactor (Saktaywin *et al.* 2006).

74 A pilot-scale experimental system was continuously operated using artificial sewage (Saktaywin
75 *et al.* 2006; Sui *et al.* 2011). The system comprises a sequencing batch reactor (SBR) with an
76 effective volume of 100 L (inner diameter: 40 cm, height: 100 cm) for simulating the A/O
77 process, a semibatch mode ozone contact reactor with an effective volume of 6 L (inner diameter:
78 10 cm, height: 100 cm) for sludge ozonation, and a phosphorus crystallization reactor with an
79 effective volume of 20 L (inner diameter: 27 cm, cylinder body height: 100 cm, and conical
80 bottom: 45 cm) for phosphorus recovery. The continuous operation of the SBR and the
81 phosphorus recovery unit was controlled by timers and water-level controllers. One operational
82 cycle of the SBR was set at 8 h (Figure S-1) and included the following stages: fill (10 min),
83 anaerobic (110 min, including mixing for 50 min and settlement for 60 min when recovering
84 phosphorus), aerobic (4 h), settlement (100 min), and draw and idle (20 min). In each SBR cycle,
85 the phosphorus recovery unit was operated in the sequence of feeding (2 min at the end of the
86 SBR anaerobic stage), reaction (33 min), settlement (50 min), and returning supernatant to the
87 SBR (1 h). Phosphorus was recovered under the conditions of a molar ratio of Ca/P 3.0 and pH
88 9.0 by adding $CaCl_2$ solution (10 g/L) and adjusting the pH using NaOH solution (1 N).
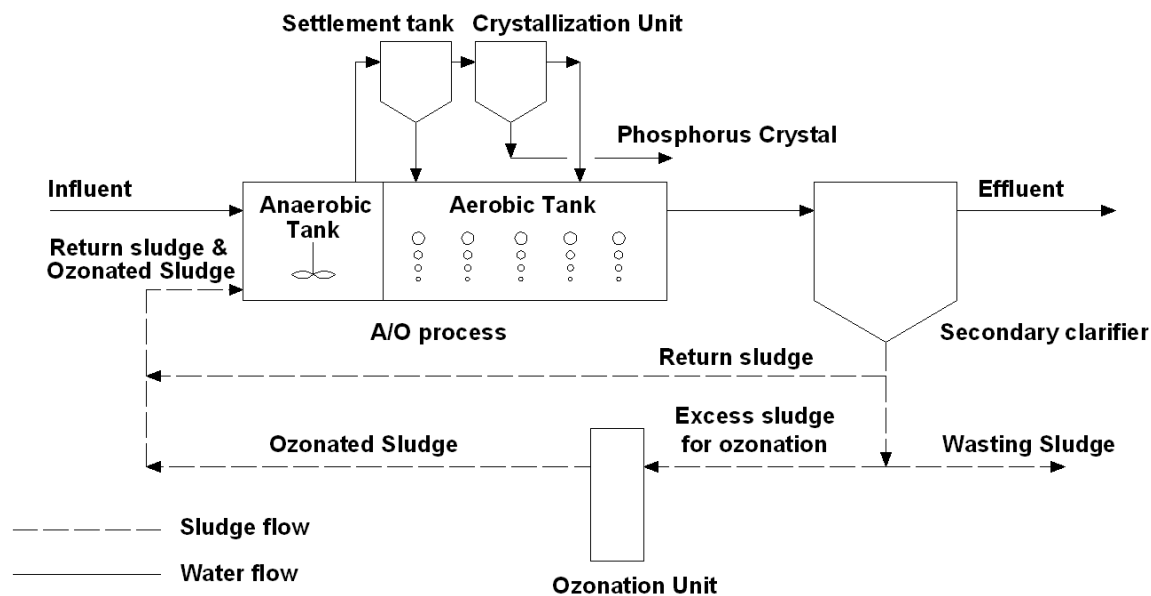
89
90 Figure 1 Configuration of the advanced SRPR process

Operational Conditions

92 The operation of the pilot-scale advanced SRPR process was divided into three phases, as shown
93 in Table 1. In Phase 1, the system was run as a conventional A/O process without excess sludge
94 reduction (i.e., without sludge ozonation) and phosphorus recovery. In Phases 2 and 3, the
95 system operated as an advanced SRPR process. The excess sludge withdrawn from the SBR was
96 maintained at 2.5 L/d in all phases. After withdrawal from the SBR, a portion of the excess
97 sludge was treated by ozone in semibatch mode (continuous flow of ozone gas and batch

3

98   treatment for sludge), and then returned to the SBR together with the influent. The flow rates of
99   the excess sludge for ozonation were 1.5 L/d in Phase 2 and 2.1 L/d in Phase 3, respectively.
100   Thus, the flow rates of wasted sludge were 1.0 L/d in Phase 2 and 0.4 L/d in Phase 3, which
101   increased the sludge reduction rate. The ozone consumption rate was set from 80 to 100 mg O_3/g
102   SS for sludge ozonation with sludge decomposition percentage of 20-30%. The flow rates of the
103   anaerobic supernatant to the phosphorus recovery reactor were 40 and 60 L/d in Phase 2 and
104   Phase 3, respectively.

105

Table 1 Operational conditions for the advanced SRPR process

| Items	Phase 1	Phase 2	Phase 3
1 Operational days [d]	67	116	97
2 SBR reactor volume [L]	100	100	100
3 Influent flow rate [L/d]	225	225	225
4 SRT [d]	10	10	10
5 Excess sludge withdrawn [L/d]	2.5	2.5	2.5
6 Ozonated sludge returned to system [L/d]	-	1.5	2.1
7 Excess sludge wasted [L/d]	2.5	1.0	0.4
8 Ozone consumption rate [mgO$_3$/gSS]	-	80–100	80–100
9 Sludge decomposed rate [%]	-	20–30	20–30
10 Flow rate to P crystallization [L/d]	-	40	60

Analytical Methods

107   The sampling frequency was once per week, and samples were taken at the end of each stage
108   (refer to Figure S-1 and Table S-1 for detailed sampling points and schedule) in the cycle from
109   8:00 to 16:00. After sampling, all soluble samples (Table S-1) were obtained by immediate
110   filtration through a 1.0 µm glass filter (Whatman® GF/B 1821-47, England). The analytical items
111   included total nitrogen (TN) in non-filtered ozonated sludge and effluent, soluble total nitrogen
112   (S-TN), ammonia (NH4), nitrite (NO2), nitrate (NO3), and total organic carbon (TOC) in filtered
113   supernatant and effluent, suspended solid (SS) in effluent, and mixed liquid suspended solid
114   (MLSS) in reactor. All analytical methods were based on Standard Methods (APHA *et al.* 2005).
115   Nitrogen oxides (NOx, sum of NO2 and NO3), soluble organic nitrogen (Org-N, S-TN minus
116   NH4 and NOx), and particulate total nitrogen (P-TN, TN minus S-TN) were calculated from the
117   measured concentrations of nitrogen species.

RESULTS AND DISCUSSION

Nitrogen Release during Sludge Ozonation

120   Excess sludge, discharged from the bioreactor, was treated with ozone. Along with particulate
121   sludge decomposition, nitrogen was released from the cells to the bulk liquid together with
122   organic carbon. The variations of the different nitrogen species in the ozonated sludge, which
123   was returned to the bioreactor together with the influent, are shown in Figure 2. For ozone
124   consumption rates of 80-100 mg O_3/g SS, approximately 35% of the particulate nitrogen in the
125   sludge was released as soluble components (S-TN). The ratio of S-TN/TN in the ozonated sludge
126   averaged 34.5% in Phase 2 and 35.6% in Phase 3. The S-TN composition, i.e., percentages of
127   soluble organic nitrogen, nitrogen oxides, and ammonia, is shown in Figure 2b. NOx was no
128   more than 2% of S-TN in most cases. Org-N averaged 70.7% and 82.2%, whereas ammonia
129   averaged 28.0% and 16.2% in Phases 2 and 3, respectively.

(a)

130

(b)
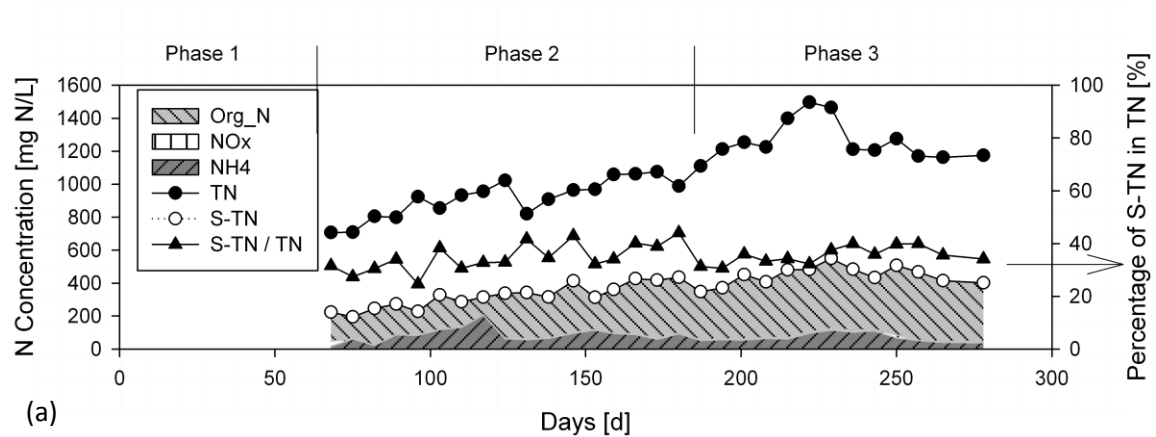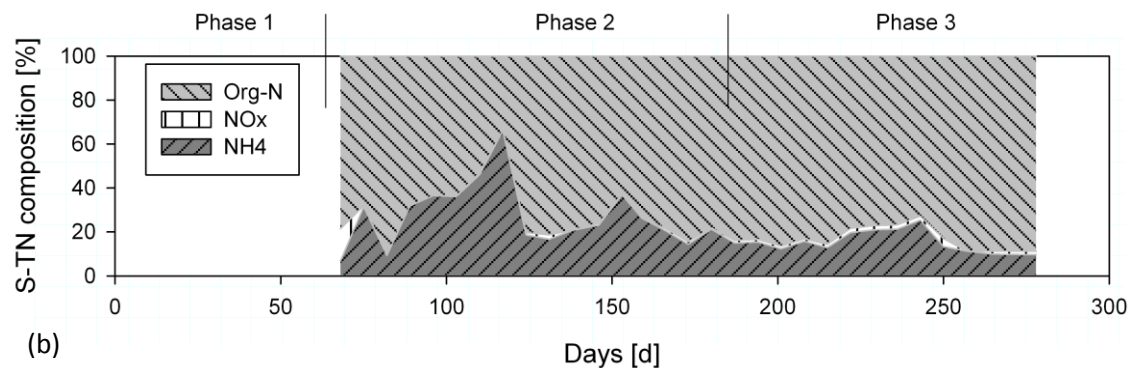
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132　　　　　　　　　　　　　　Figure 2 Nitrogen species in the ozonated sludge.
133　　　　(a) Concentration of each nitrogen species; (b) Percentage of each nitrogen species in S-TN
134　　　Note: No data for phase 1 because it's run as the conventional A/O process and no sludge ozonation was
135　　　combined; Org-N: organic nitrogen; NOx: nitrogen oxides; NH4: ammonia; TN: total nitrogen; S-TN:
136　　　soluble total nitrogen

137　　　Along with sludge reduction, mixed liquor suspended solids in the bioreactor increased
138　　　incrementally (Figure S-2). To maintain the same ozone consumption rate (80-100 mg O_3/g SS),
139　　　and therefore the same sludge decomposition rate (20%-30%), the reaction time of the sludge
140　　　ozonation increased under the same inlet ozone concentration. It was found that the ammonia
141　　　concentration in the ozonated sludge increased gradually in Phase 2 (68–117 days in Figure 2a).
142　　　To reduce the total reaction time of the sludge ozonation, the inlet ozone concentration was
143　　　increased from 30 to 80 mg O_3/L on the 124th day. The reaction time of the sludge ozonation
144　　　decreased from 29 h to 11 h, approximately. It was found that S-TN in the ozonated sludge
145　　　remained at the same level (approximately 330 mg N/L) because the sludge decomposition rate
146　　　did not change under the same ozone consumption rate. However, the S-TN composition in the
147　　　ozonated sludge changed sharply. The ammonia concentration decreased from 207 mg N/L (66.1%
148　　　of S-TN) to 63 mg N/L (18.7% of S-TN), whereas the Org-N increased from 105 mg N/L (33.7%
149　　　of S-TN) to 271 mg N/L (80.3% of S-TN). The results suggest that the composition of the
150　　　released nitrogen was affected by the inlet ozone concentration and reaction time. For the same
151　　　ozone consumption rate, the lower inlet ozone concentration and longer ozonation time
152　　　contributed to the oxidation of more organic nitrogen to ammonia.

153 It was well documented that raw excess sludge decomposed to suspended micro particles, then
154 oxidized to soluble COD, and finally mineralized to carbon dioxide gradually during sludge
155 ozonation (Chu *et al.* 2009). However, for the transformation of nitrogen, most researchers have
156 reported that organic nitrogen was the main oxidation product during sludge ozonation (Zhao *et*
157 *al.* 2007; Naso *et al.* 2008); further oxidation to ammonia has seldom been reported. Cesbron *et*
158 *al.* (2003) investigated the competition for ozone between the soluble compounds and solid
159 particles and corresponding chemical reaction rates. Nishijima *et al.* (2003) concluded that long-
160 term ozonation was not effective in producing biodegradable organic forms because most of the
161 ozone was used to oxidize the biodegradable products generated in the early stage of ozonation.
162 Similar to the oxidation of organic carbon, the results in this study suggest that organic nitrogen
163 does not always increase and may oxidize to ammonia. Under the condition of the same total
164 ozone consumption rate, the lower ozone inlet concentration and longer ozonation time
165 contributed to the oxidation of more organic nitrogen to ammonia. This should be avoided to
166 reduce the operational costs of sludge ozonation because the oxidation of organic nitrogen to
167 ammonia by ozone gas is much more costly than oxidation in the subsequent biological treatment
168 process. The above results were derived solely from the observations of long-term operation of
169 the advanced SRPR process. More detailed studies are required to determine the chemical
170 reaction rates between nitrogen compounds and ozone, as well as the effects of physical mixing
171 and bubble size.

172 **Nitrogen Removal by Biological Process**

173 Higher ozone inlet concentration and shorter ozonation time result in organic nitrogen release
174 mainly during sludge ozonation. However, it is moot whether such released organic nitrogen can
175 be effectively and steadily removed in the A/O process. As shown in Figure 3a, the average S-
176 TN in the effluent ranged from 7.5 to 8.5 mg N/L in each phase. Among nitrogen species, the
177 average Org-N concentration in the effluent (Figure 3b) increased from 0.54 mg N/L (7.3% of S-
178 TN) in Phase 1 to 0.78 mg N/L (9.2% of S-TN) in Phase 2, and 1.0 mg N/L (12.7% of S-TN) in
179 Phase 3. The average total organic carbon (TOC) in the effluent was found to increase from 3.3
180 mg C/L in Phase 1 to 7.9 mg C/L in Phase 2 and 10.4 mg C/L in Phase 3 (Figure 3b). It has been
181 reported that soluble organic carbon in the effluent increased because of the non-biodegradable
182 fraction from sludge ozonation (Chu *et al.* 2009). For ozone consumption rates of 30-40 mg O_3/g
183 SS, 25% of the soluble organic in the ozonated sludge was found to be refractory matter
184 (Saktaywin 2005). Refractory organic matter during sludge ozonation originated from the
185 disintegration of activated sludge and mainly consisted of proteins and sugar moieties (Dignac *et*
186 *al.* 2000). However, there are few reports in the literature for increasing organic nitrogen in the
187 effluent. The increase in organic nitrogen in the effluent was attributed to with the same
188 mechanism as the increase in refractory organic carbon. Nitrogen in amide groups generated by
189 sludge ozonation was refractory to the treatment (Dignac *et al.* 2000).

190 Almost all inorganic nitrogen in the effluent was nitrate nitrogen (Figure 3a), which suggest
191 good nitrification. However, an unusually high level of ammonia was detected in the effluent
192 (10.8 mgN/L, full nitrification was not obtained), which coincided with the change in operational
193 conditions of sludge ozonation on the 124th day. The boxplots in Figure 3c show three outliers
194 for ammonia (more than 0.1 mg N/L) in the observations. According to the measured Org-N and
195 NH4 in the ozonated sludge (as shown above) and influent (2.65 g N/d for NH4 and 1.85 g N/d
196 for Org-N) as well as mixed liquor volatile suspended solids (MLVSS, 3.18 mg/L on the 124th

197 day), Org-N loading increased from 2.81 to 3.16 mg N/g VSS (12.4%) while NH4 decreased
198 from 4.14 to 3.84 mg N/g VSS (-7.3%). This shock loading of nitrogen was assumed responsible
199 for the failure of full nitrification. However, after only three weeks, the expected performance of
200 the advanced SRPR process was re-established and full nitrification was restored. The rapid
201 restoration of nitrification suggests that biological treatment is adaptive and capable of
202 converting Org-N to nitrate; moreover, avoiding conversion during sludge ozonation is feasible.

203

(a)

204

(b)

205

(c)
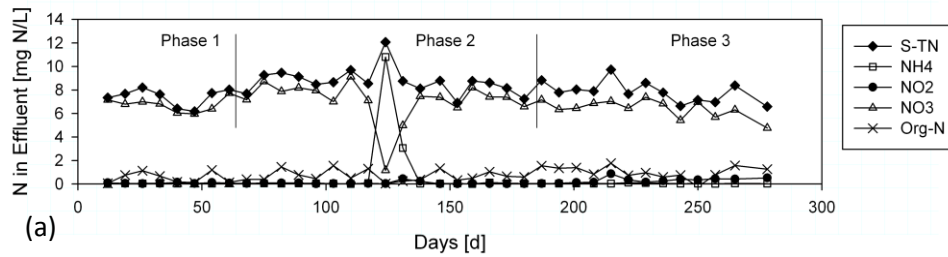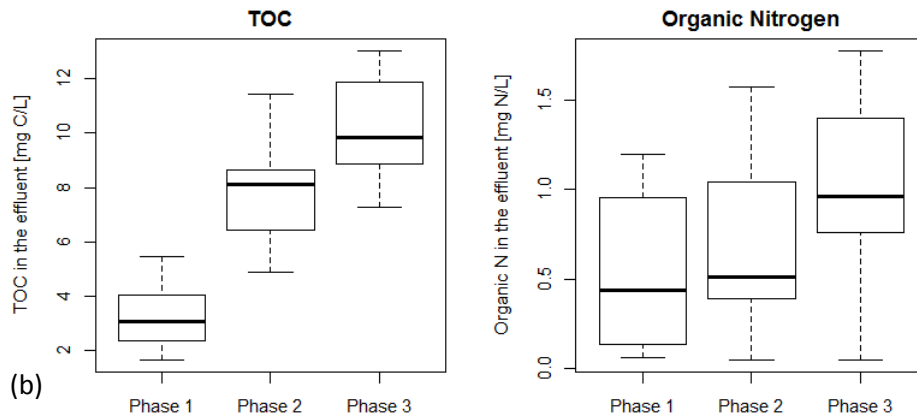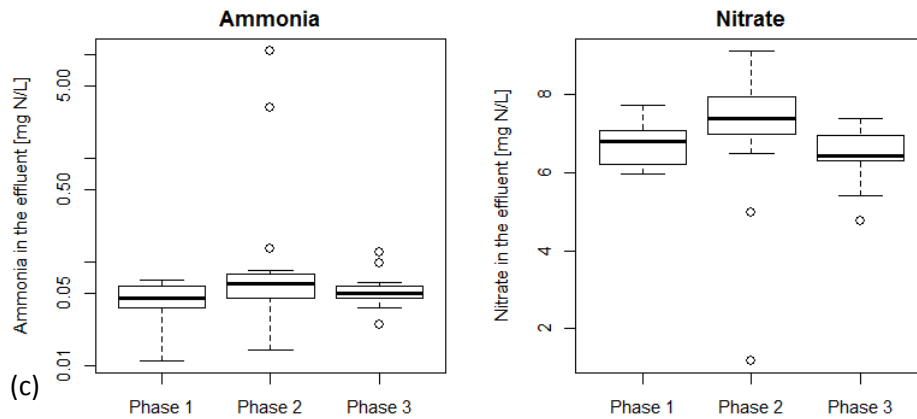
206 Figure 3 (a) Concentration of nitrogen species in the effluent;
207 (b) Boxplot of TOC and organic nitrogen in the effluent in each operational phase;
208 (c) Boxplot of ammonia and nitrate in the effluent in each operational phase.

7

Mass Balance of Nitrogen in the Advanced SRPR Process

The mass balance of nitrogen in the advanced SRPR process is shown in Figure 4. Because ozonated sludge was cycled back to the bioreactor internally, the nitrogen inflow to the system was influent only, whereas the outflow included nitrogen discharged by the excess sludge, nitrogen in the effluent, nitrogen precipitated in the phosphorus crystallization (P-cry) reactor and nitrogen removed by denitrification (N_2). On the basis of the experimental results, the particulate nitrogen in the wasted excess sludge, the effluent, and the loss in the P-cry reactor were calculated by the corresponding SS concentrations and measured nitrogen contents in SS (mg P-TN/g SS). The soluble nitrogen in effluent came from S-TN measurements.
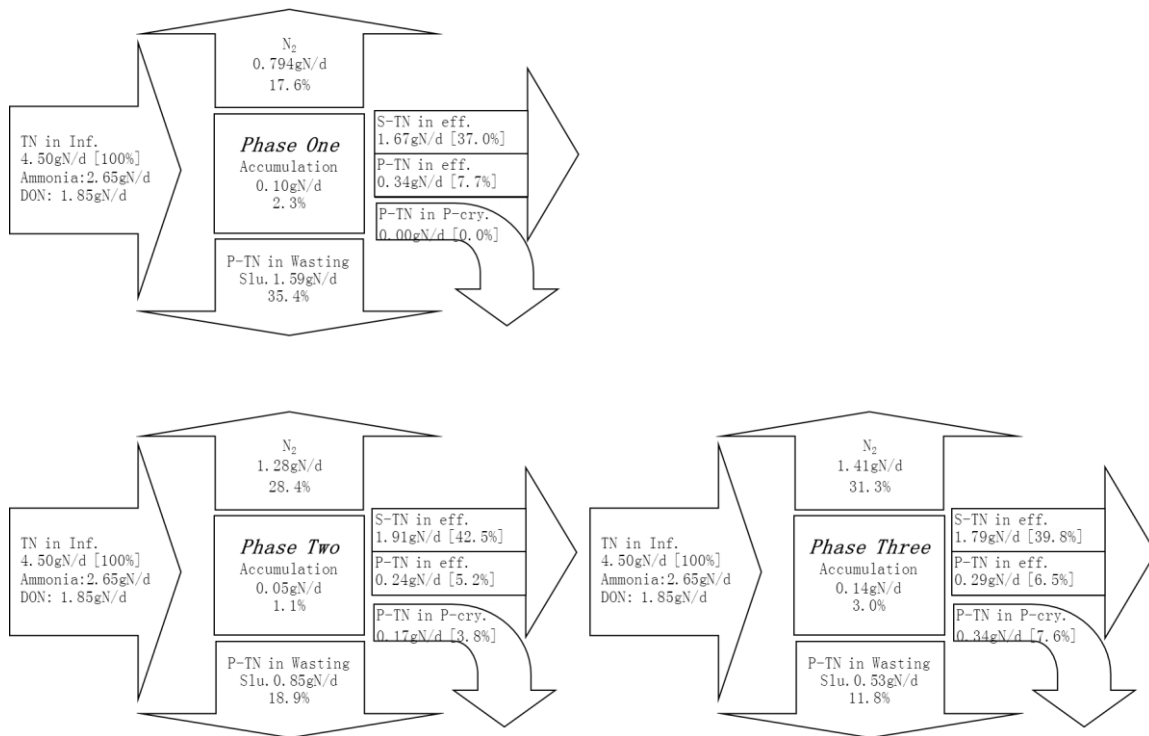
Figure 4 Mass balance for nitrogen in the advanced SRPR process.
(TN: total nitrogen; DON: dissolved organic nitrogen; P-TN: particulate total nitrogen;
S-TN: soluble total nitrogen; P-cry: Phosphorus crystallization unit; Inf.: influent; eff.: effluent)

According to the mass balance calculations for nitrogen, the particulate nitrogen in wasted sludge decreased from 35.4% in Phase 1 to 18.9% in Phase 2 and 11.8% in Phase 3, corresponding to the increasing reduction rate of excess sludge. Nitrogen gas generated by denitrification increased from 17.6% in Phase 1 to 28.4% in Phase 2 and 31.3% in Phase 3, which indicated the increase in nitrogen removal. TN in the effluent accounted for 44.7%, 47.7%, and 46.3% in the three operational phases, respectively. The results indicated that the nitrogen in the effluent did not clearly increase with increasing reduction rate of excess sludge. The reduced nitrogen in the excess sludge mainly transformed to nitrogen gas by denitrification after combined A/O process with sludge ozonation.

By sludge ozonation, organic carbon and nitrogen were released from the biomass owing to sludge disintegration. The released organic carbon was probably reused by microorganisms and a

fraction of the organic carbon transformed to carbon dioxide. With the same mechanisms, the released nitrogen from the biomass could also be oxidized from organic nitrogen or ammonia to nitrates. From this perspective, the combined biological process with sludge ozonation has a high nitrogen removal potential. Furthermore, denitrification is often limited by the carbon source in an actual sewage treatment plant; it has been proven that organics released from sludge ozonation can serve as the carbon source for denitrification (Ahn *et al.* 2002; Cui & Jahng 2004; Dytczak *et al.* 2007). It has been reported that the denitrification rate improved up to 20% because of the additional carbon release by ozonation at an ozone dosage of 0.08 g O_3/g TSS (Dytczak *et al.* 2007), which is consistent with the results obtained in this study.

CONCLUSIONS

Nitrogen behavior during sludge ozonation and subsequent biological removal was examined via observations of long-term operation of a lab-scale experimental system. The main conclusions are as follows:

1. For ozone consumption rates of 80-100 mg O_3/g SS, 35% particulate nitrogen was released as the soluble components. Nitrogen release during sludge ozonation strongly depended on the operational conditions. At the same ozone consumption rate, the lower inlet ozone concentration and longer ozonation time contributed to the oxidation of more soluble organic nitrogen to ammonia. This should be avoided to reduce the operational costs of sludge ozonation.

2. Full nitrification was restored in three weeks after the shock loading of nitrogen, caused by the change in operational conditions during sludge ozonation. The rapid restoration of nitrification suggests that biological treatment is adaptive and capable of converting Org-N to nitrate; furthermore, avoiding conversion during sludge ozonation is feasible.

3. Assuming the same mechanisms as the increase in refractory organic carbon, soluble organic nitrogen in the effluent was found to increase owing to sludge ozonation.

4. After combining the A/O process with sludge ozonation, the mass balance for nitrogen showed that nitrogen in excess sludge decreased with increasing sludge reduction rate. Nitrogen removal by denitrification clearly increased, whereas nitrogen in the effluent did not clearly increase. The reduced nitrogen in excess sludge mainly transformed to nitrogen gas by denitrification.

Based on above findings, it's concluded that combination of sludge ozonation into A/O or A/A/O process is a feasible and promising technology for sludge reduction and nitrogen removal simultaneously.

ACKNOWLEDGEMENTS

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