



The threshold of influent ammonium concentration for nitrate over-accumulation in a one-stage deammonification system with granular sludge without aeration

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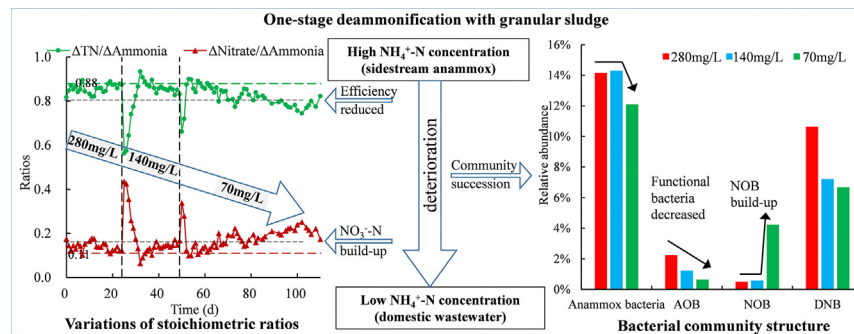
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HIGHLIGHTS

- The threshold of influent ammonium for stable deammonification was 70 mg/L.
- Obvious nitrate accumulation and NOB build-up happened with 70 mg/L ammonium.
- Stable performances were achieved in 280 or 140 mg/L of ammonium without aeration.
- DNB, AOB and anammox bacteria declined after lowering influent ammonium.
- *Chloroflexi*, *Proteobacteria*, and *Planctomycetes* dominated in three ammonium levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Low-strength ammonium is still a challenge for the mainstream deammonification because of nitrate over-accumulation. In this study, the threshold of influent ammonium concentration of one-stage deammonification system with granular sludge was investigated, by stepwise decreasing influent ammonium from high concentrations (280 mg/L to 140 mg/L) to the low concentration (70 mg/L) in 108 d at 32 °C without aeration. Results showed that, under 70 mg/L $\text{NH}_4^+\text{-N}$, $\Delta\text{NO}_3^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ ratio increased to 0.2, deviated from the theoretical value of 0.11, with ammonium and TN removal efficiencies of 91% and 71%, respectively. However, under both high ammonium concentrations (280 mg/L and 140 mg/L), nitrate production stabilized at only 13%. *Chloroflexi*, *Planctomycetes* and *Proteobacteria* contributed >70% of the communities under all three ammonium concentrations. As influent ammonium decreasing, the relative abundances of bacteria for anammox, aerobic oxidizing and denitrifying decreased, while NOB (nitrite oxidizing bacteria) abundance increased greatly. So 70 mg/L was the threshold of influent ammonium concentration for stable deammonification without organic influent. It was the decrease of functional bacteria and overgrowth of NOB that worsen the deammonification performance under low-strength ammonium.

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1. Introduction

The combination of partial-nitritation and anammox (also called PN/A or deammonification) is a promising biological nitrogen removal process for autotrophic elimination of nitrogen with low COD/TN

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(Chemical Oxygen Demand/Total Nitrogen). Compared to the conventional nitrogen removal via nitrification-denitrification process, deammonification process allows about 60% reduction in aeration-related costs without external organic carbon. Meanwhile, the costs of excess sludge handling and transportation are cut down by 90%, and the emission of greenhouse gas of N_2O is also reduced (Salmistraro et al., 2017). Nowadays, over 88% of all full-scale anammox applications are operated as one-stage systems in order to reduce the capital costs (Lackner et al., 2014). Deammonification process has been considered as efficient, economic, and environmentally friendly.

To date, most of reported one-stage deammonification applications performed well under temperature higher than 25 °C and influent ammonium concentration above 100 mg N/L (Van Hulle et al., 2010). And one-stage deammonification has been successfully implemented in elimination of nitrogen from warm wastewaters with high ammonium and low COD/N, including sludge digester supernatant in sidestream treatment and other industrial wastewaters (Joss et al., 2009; Rosenwinkel and Corneliuss, 2005; Tokutomi et al., 2011; Wett, 2007; Zhang et al., 2016a). More than 200 full-scale facilities were working successfully in Europe, Asia and North America until 2014 (Lackner et al., 2014). However, sidestream nitrogen is only 15–20% of the influent in wastewater treatment plants (WWTPs). Next, applying one-stage deammonification to the main line of ammonium removal in municipal wastewater treatment (mainstream deammonification) has recently become a prioritized research area.

Mainstream deammonification allows the decoupling of carbon and nitrogen removal. Because the biodegradable organic carbon is not anymore necessary to heterotrophic denitrify, energetic CH_4 produced by the previous anaerobic digestion is supposed to increase by about 50% (Carrère et al., 2010). Therefore, total electrical energy consumption can decrease by 30–40% with biogas utilization (Fux and Siegrist, 2004). WWTPs with anammox in the mainstream would yield 24 watt hours per person per day (Wh/p/d), compared to 44 Wh/p/d consumption in conventional treatment (Siegrist et al., 2008). This would get the energy neutral/positive in the WWTPs.

One of the main challenges of mainstream deammonification is the low ammonium concentration of domestic wastewater. Influent NH_4^+ -N of as low as 30–90 mg/L triggers the over-development of NOB, which leads to excessive oxidation of nitrite to nitrate and therefore impedes the nitrogen removal through anammox pathway. Meanwhile, low ammonium content demand low HRT (Hydraulic Retention Time) and low SRT (Sludge Retention Time) to supply enough NLR (Nitrogen Loading Rate), therefore the net biomass production of functional bacteria was low because of continuous biomass washout. Under low ammonium, biofilm or granular biomass with good retaining ability are better choices (Cao et al., 2017). By the uncoupled SRT of flocculent biomass (often AOB, NOB or heterotrophic bacteria dominated) to the attached biomass (biofilm or granule, often anammox bacteria dominated), the functional anammox bacteria and AOB can be selectively enriched under controlled floc SRT (Zhang et al., 2016a). The bacterial community dynamics between high concentrated and diluted substrate (mainly ammonium) has great significant. Persson recently evaluated the community structures of PN/A biofilms at decreasing substrates (ammonium and COD) under low 13 °C, and concluded that changes of bacteria community were quite small, including heterotrophic bacteria (43–50% of total sequences), although the nitrate production of as high as 15% to 58% were over-produced (Persson et al., 2016). From stable performance under high concentration of influent ammonium, to deteriorated performance with nitrate over-accumulation under low concentration of influent ammonium, there should be a threshold of influent ammonium concentration that caused the out-control of NOB growth and disturbed the stable performance of one-stage deammonification system, which was rare concluded.

This study aimed to investigate the threshold of influent ammonium concentration disturbing the stable performance of one-stage deammonification system. A granular deammonification system in a

lab-scale EGSB (Expanded Granular Sludge Bed) was fed with synthetic ammonium medium without organic carbon, which was stepwise decreased from high ammonium (280 mg/L and 140 mg/L) to low ammonium (70 mg/L) to simulate the ammonium concentration from sidestream treatment (such as anaerobic digestion supernatant) to mainstream treatment (such as domestic wastewater). No aeration behavior was implemented in this deammonification process during whole operation. The nitrogen removal efficiencies and bacterial community dynamics undergoing through the decreasing ammonium stages were analyzed. The link between bacterial dynamics and ammonium concentrations was evaluated to analyze the deterioration under the threshold of influent ammonium concentration.

2. Materials and methods

2.1. The reactor configuration

In this study, a typical lab-scale EGSB reactor made of polymethyl methacrylate was employed for the operation of one-stage deammonification process with granular sludge, with total and working volumes of 1.3 L and 0.5 L, respectively. The diameter was 4 cm, with the height/diameter ratio of 10 (Fig. S1) (Wang and Gao, 2016).

This reactor was started-up by inoculating only anammox granular biomass (Wang and Gao, 2017). And this one-stage deammonification process had stably operated >2 months, with ca. 30 g VSS/L of sludge.

2.2. The experimental design and reactor operation

This study could be divided into three stages based on the stepwise decreasing influent NH_4^+ -N concentrations (280 mg/L, 140 mg/L, and 70 mg/L) (Table 1).

During the entire operation, no any aeration equipment and behaviors were implemented. Therefore, the DO (Dissolved Oxygen) values were always below the detection of our equipment (<0.08 mg O_2 /L). The pH in the reactor was maintained around 8.1 ± 1.0 by elevated the influent pH. Temperature was maintained around 33 ± 1 °C through a warm water cycle in the water jacket. A blackout cloth covered the reactor. The recirculation ratio (recirculated flux/influent flux) was between 111 and 250, which brought the upflow velocity of ca. 15 m/h to provide high enough shear stress for granules.

In the theoretical deammonification reaction by cooperating between AOB and Anammox, nitrate and nitrogen gas can only be conducted by anammox reaction, so two theoretical molar ratios exist: $\Delta TN/\Delta NH_4^+$ -N (removed TN to removed ammonium) and $\Delta NO_3^-/N/\Delta NH_4^+$ -N (produced nitrate to removed ammonium), which are 0.88 and 0.11, respectively (Eq. (1)). The two stoichiometric ratios can be used to estimate the quality and contamination of deammonification process. When $\Delta NO_3^-/N/\Delta NH_4^+$ -N is 0.11, a theoretical deammonification system will obtained its best potential TN removal efficiency of 88%. If $\Delta NO_3^-/N/\Delta NH_4^+$ -N ratio is above 0.2, the theoretical TN removal efficiency will below 80%, which suggested serious nitrate over-accumulation and >15 mg/L NO_3^- -N for treating domestic wastewater as emission standards of China or EU (Table 2). So we define that the influent ammonium concentration reached its threshold

Table 1
The operation parameters in three stages of deammonification process.

Operational set-up	Stage I	Stage II	Stage III
Time (day)	1–24	25–49	50–108
Inf. NH_4^+ -N (mg/L)	280	140	70
Inf. NH_3 -N (mg/L) (pH 8.6)	96	48	24
NLR (g N/L/d)	1.08	0.54	0.62
HRT (h)	6.25	6.25	2.78
Temperature	33 ± 0.1		
Effluent pH	8.1 ± 0.1		
DO	No aeration, always under detection		

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