



# Start-up of single-stage partial nitrification-anammox process treating low-strength swage and its restoration from nitrate accumulation



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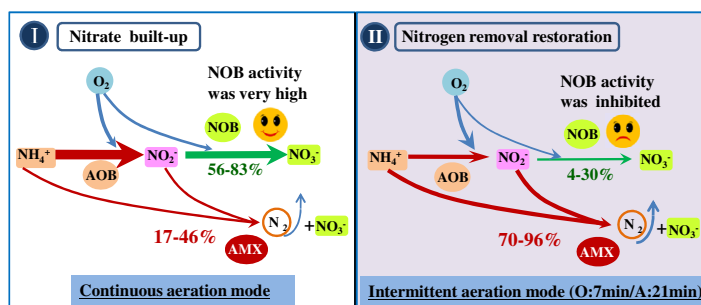
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## HIGHLIGHTS

- Intermittent aeration restored single-stage PN/A process from nitrate build-up.
- NOB accumulated under continuous aeration mode, *Nitrospira* was the dominant NOB.
- Reducing DO to  $0.17 \pm 0.08$  mg/L was failed to inhibited the NOB activity.
- Sewage PN/A reactor was quickly started up by inoculating anammox sludge.

## GRAPHICAL ABSTRACT

The conversion pathway of  $\text{NH}_4^+$  in deterioration and restoration periods.



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## ABSTRACT

A single-stage partial nitrification-anammox (PN/A) reactor treating low-strength swage was operated for 288 days to investigate the recovery of nitrogen removal from nitrate accumulation. The reactor was quickly started up by inoculating anammox sludge. However, nitrite oxidizing bacteria (NOB) abundance gradually increased on day 25, leading to high effluent nitrate concentration. Two strategies were executed to control the effluent nitrate. In strategy I, dissolved oxygen (DO) concentration was kept low ( $0.17 \pm 0.08$  mg/L), but nitrate production increased from 4.71 to 38.18 mg-N/L. In strategy II, intermittent aeration operation mode (aeration 7 min/anoxic 21 min) was adopted, which significantly lowered the nitrate concentration to 1.3 mg-N/L, indicating the NOB was inhibited. The high nitrogen removal rate of 73 mg-N/(L·d) was achieved. The evolution of bacterial activity and abundance verified the changes of the nitrogen removal performance and proved the intermittent aeration strategy could successfully solve the problem of nitrate build-up in the PN/A process.

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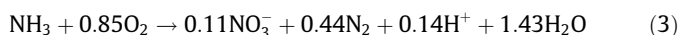
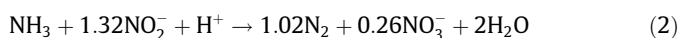
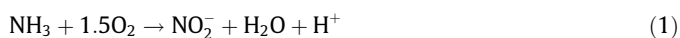
## 1. Introduction

The single-stage partial nitrification-anammox (PN/A) combines partial nitrification with anammox in a single reactor. Part of ammonium in wastewater is first oxidized to nitrite by ammonium

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oxidizing bacteria (AOB) (Eq. (1)) and then the remaining ammonium and nitrite are converted to nitrogen gas by anammox bacteria (Eq. (2)). The overall PN/A reaction of these autotrophic nitrogen removal processes is summarized in Eq. (3) (Sliemers et al., 2002). Nitrogen removal with PN/A saves about 60% of the aeration, 90% of the sludge handling and transport, and 100% of the organic carbon addition compared to conventional nitrification/denitrification (Mulder, 2003).



The single-stage PN/A process has been mainly studied for the treatment of ammonium-rich wastewater. Recently, the feasibility of single-stage PN/A for low ammonium sewage was verified (De Clippeleir et al., 2013, 2011; Hu et al., 2013). However, there are still many problems for its application in sewage treatment. Nitrate build-up is the critical issue, which leads to a high nitrogen concentration in effluent and deteriorates nitrogen removal performance. Nitrate build-up is caused by the enrichment of NOB. Because nitrite could be rapidly consumed by NOB, the anammox bacterial activity was limited due to an inadequate supply of nitrite and nitrogen removal efficiency dropped in PN/A processes.

Nitrate accumulation was found in anammox reactors treating low strength wastewater (Malovany et al., 2015; Lotti et al., 2015). Over 50% of 100 full-scale plants treating ammonium-rich wastewater globally have the problem of nitrate build-up (Lackner et al., 2014; Jardin and Hennerkes, 2012). To solve nitrate accumulation in PN/A process, several strategies have been used, such as re-inoculating with the anammox biomass devoid of NOB and adding chemicals (e.g. hydroxylamine) to selectively inhibit NOB (Joss et al., 2011; Wang et al., 2015). But these strategies need high operational cost due to long cultivating period of anammox bacteria and high cost of chemicals. Low DO concentration, intermittent aeration and removal of flocculent sludge have also been applied to solve nitrate accumulations (Lackner et al., 2014). At low DO aeration, nitrite easily accumulated due to higher growth rate of ammonium oxidizing bacteria (AOB) than that of NOB (Tokutomi, 2004). Shortening aeration duration to 8 min was effective at inhibiting the NOB activity (Jardin and Hennerkes, 2012).

Until now, PN/A process has been mainly studied for treating ammonium-rich wastewater. The effectiveness of the above mentioned strategies for PN/A process treating ammonium-low sewage has not been investigated yet. In fact, it is more difficult to repress NOB in sewage treatment due to the lack of the inhibition factors such as high free ammonium (FA) and free nitrite (FNA) (Yang et al., 2007; Blackburne et al., 2008). In addition, the relationships among AOB, NOB and anammox bacteria in PN/A reactors remain unknown. Therefore, the purpose of this study was to conduct a single-stage PN/A process that treating real sewage with low ammonium concentration, and explore the effective strategies for restoring the nitrogen removal of the deteriorated single-stage PN/A process caused by nitrate build-up. Two strategies were implemented in order to inhibit NOB: (1) reducing the DO concentration to  $0.17 \pm 0.08$  mg/L; and (2) an intermittent aeration mode with 7 min aeration/21 min anoxic period. Sludge retention time (SRT) control was adopted as the supplement for better eliminating the NOB. The evolutions of AOB, NOB and anammox bacteria were characterized using the quantitative PCR (qPCR) assays. The bacterial activity was determined for better understanding of the PN/A restoration mechanism.

## 2. Materials and methods

### 2.1. Experimental setup

The sequencing batch reactor (SBR) reactor (plexiglass, working volume: 10 L) used for the PN/A process was equipped with an air-compressor for aeration, and a mechanical stirrer for mixing during the feeding and reaction stages. The cycle duration was 6 h, consisting of 4 min feeding, 322 min aeration, 30 min settling and 4 min decanting. During the intermittent aeration phase, air supply was adjusted intermittently using a time relay control system to turn on and turn off the air-compressor alternatively and create many cycles of aerobic (7 min) and anoxic (21 min) conditions. At the feeding stage, 5 L pre-treated sewage was added to the reactor. The temperature was maintained at  $32 \pm 1$  °C.

### 2.2. Influent and seed sludge

The influent was the real sewage (collected from the residential area of Beijing University of Technology (Beijing, China)) that was pretreated to remove chemical oxygen demand (COD). The real domestic wastewater was pretreated in a SBR, which was operated using a traditional mode: filling-aeration-settle-decant (2 h), the aeration of each cycle duration was 40 min to remove chemical oxygen demand (COD), the SRT was controlled at 10 days to wash-out AOB. Both the short aeration time and SRT could prevent the ammonium from being degraded. The characteristics of the feeding pretreated domestic wastewater to the single-stage PN/A reactor were as follows: ammonium 51.2–67.5 mg-N/L, nitrite 0–5.2 mg-N/L, nitrate 0–0.4 mg-N/L, soluble COD 41.4–70.2 mg/L, BOD<sub>5</sub> 3.5–10.8 mg/L, SS 35–54 mg/L and pH 7.56–7.71. The seed anammox granule sludge was taken from a pilot-scale PN/A reactor treating ammonium-rich wastewater in Beijing Gaobeidian Sewage Treatment Plant (Beijing, China) (Zhang et al., 2015). The seed partial nitrification sludge was obtained from a pilot scale SBR system treating sewage at the Beijing University of Technology (Beijing, China). The seed sludge contained 8 L partial nitrification sludge with a MLSS of 3350 mg/L and 2 L anammox granule sludge with a MLSS of 11,000 mg/L. The initial MLSS of single-stage PN/A reactor was 4898 mg/L. A wet-sieving method with a sieve of 200 μm was used to make sure the anammox particles were larger than 200 μm.

### 2.3. Experiment process

Experimental process was divided into four phases based on different aeration modes. In Phases I and II, the reactor was operated under continuous aeration. In Phases III and IV, the reactor was operated under intermittent aeration. In Phases I–IV, 100 ml, 250 ml, 200 ml and 125 ml sludge was discharged daily, respectively. Anammox granules greater than 200 μm were reclaimed from the discharged sludge using a wet-sieving method with a sieve of 200 μm to ensure the anammox biomass in reactor. Since only floc sludge was discharged, the “sludge retention time” in our manuscript was floc sludge retention time. The main experimental conditions and operational parameters under different phases were summarized in Table 1.

### 2.4. Bacterial activity

In order to investigate the maximum activity of AOB, NOB and anammox bacteria, a series of batch experiments were conducted as previously described (Moussa et al., 2003). The activated sludge samples were washed three times by deionized water, and then injected into a 1 L flask to measure AOB and NOB activity. The aver-

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