Inhibitive effects of chlortetracycline on performance of the nitritation-anaerobic ammonium oxidation (anammox) process and strategies for recovery

Hong Yao*, Huayu Li, Jing Xu, Lushen Zuo

Beijing Key Laboratory of Aqueous Typical Pollutants Control and Water Quality Safeguard, Department of Municipal and Environmental Engineering, Beijing Jiaotong University, Beijing 100044, China

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ABSTRACT

The short- and long-term effects of chlortetracycline (CTC) on the nitritation- anaerobic ammonium oxidation (anammox) process were evaluated. The half maximal inhibitory concentration of CTC in the batch tests of the nitritation-anammox process was 278.91 mg/L at an exposure time of 12 hr. The long-term effects of CTC on the process were examined in a continuous-flow nitritation-anammox reactor. Within 14 days, the nitrogen removal rate significantly decreased from 0.61 to 0.25 kg N/m^3/day with 60 mg/L CTC in the influent. The performance suppressed by CTC barely recovered, even after CTC was removed from the influent. Furthermore, the inhibition of CTC also reduced the relative abundance of ammonium oxidizing bacteria (AOB) and anaerobic ammonium oxidizing bacteria (AnAOB) in the reactor, resulting in both a decreased amount of and an imbalance between AOB and AnAOB. When fresh anammox sludge was reseeded into the nitritation-anammox reactor, the nitrogen removal rate recovered to 0.09 ± 0.03 kg N/m^3/day.

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Introduction

The anaerobic ammonium oxidation (anammox) process is an innovative and promising alternative for the treatment of nitrogen-rich wastewater, because it is environmentally friendly and cost effective (Loosdrecht and Damir, 2014). When the anammox process is applied to wastewater treatment, nitration is the preliminary step for producing nitrite, after which anaerobic ammonium oxidation bacteria (AnAOB) directly oxidize ammonia with nitrite to produce nitrogen gas and nitrate (Strous et al., 1998a). To date, the nitritation-anammox process has been applied to nitrogen-rich wastewater, such as sludge digester liquor, tomato processing effluent, and landfill leachate (Nhat et al., 2014; van der Star et al., 2007). However, application of this process has been restricted by the growth

characteristics of AnAOB and the widespread inhibitory factors that exist in ammonium–rich wastewater, such as antibiotics, heavy metals, and sulfide (Jin et al., 2013; Liu and Horn, 2012; Tang et al., 2011).

Antibiotics are extensively used in human and veterinary medicine, and high antibiotic concentrations have been detected in aquatic environments, such as pharmaceutical wastewater, sewage treatment plants, and surface and ground water (Baquero et al., 2008). Chlortetracycline (CTC) is a broad-spectrum antibiotic that is mass produced and widely used for animal husbandry, aquaculture, and human disease control because it is active against a broad range of Gram-positive and Gram-negative bacteria (Alvarez et al., 2010). The widespread use of CTC has led to its presence in aquatic and soil environments, such as surface water (122.3 ng/L) (Tong et al., 2014) and wastewater

* Corresponding author. E-mail: hyao@bjtu.edu.cn (Hong Yao).

(1.8 ± 0.5 mg/L) (Hou et al., 2016). CTC residue can have several adverse effects, such as inhibition of microbial activity and growth (Liu et al., 2015; Zielezny et al., 2006) and changes in the microbial community structure (Stone et al., 2011). When AnAOB are exposed to antibiotics, their activity can be significantly inhibited; thus, their abundance does not satisfy the biomass requirement. Moreover, anammox bacteria have a slow growth rate and cellular yield (Zhang et al., 2015), indicating a potential long-term recovery period when inhibited by influent wastestream. Thus, understanding the inhibition of anammox and subsequent recovery are important to its application and long-term operations.

To date, there have been several studies of antibiotic inhibition of the anammox process. Fernandez et al. (2009) demonstrated a decrease of specific anammox activity (SAA) by 40%, 60%, and 80% with chloramphenicol concentrations of 250, 500, and 1000 mg/L, respectively, in batch tests. The SAA decreased by 30%, 40%, 60%, 60%, and 80% with the addition of 100, 200, 250, 500, and 1000 mg/L tetracycline hydrochloride, respectively, to influent samples. The half maximal inhibitory concentration (IC_{50}) of oxytetracycline and sulfathiazole on SAA after a 24 hr exposure was 1100 and 650 mg/L, respectively (Lotti et al., 2012a). Yang et al. (2013a) reported that the IC_{50} was 517.5 mg/L during the batch tests. To the best of our knowledge, few studies have investigated changes in the performance of the anammox process in the presence of CTC. Most studies have focused on the inhibitory effects of antibiotics on this process. Because more than 90% of the full-scale nitritation-anammox system is single stage (Lackner et al., 2014; Wang et al., 2014), the effects of antibiotics on the single-stage anammox process require more research.

In this study, the nitritation-anammox process was established to investigate the effects of CTC on the nitrogen removal rate (NRR). The objectives were to evaluate the effects of CTC on nitrogen removal performance and functional bacteria variation and to investigate recovery strategies after inhibition of CTC.

1. Materials and methods

1.1. Inoculated anammox biomass and wastewater

Three forms of biomass, including biofilm on sponge cubes, flocculent sludge, and granular sludge were collected from a nitritation-anammox pilot-scale reactor (110 cm × 10 cm × 60 cm) at Beijing Jiaotong University (Beijing, China), and used as inoculates for the batch and continuous nitritation-anammox reactor. The reactor had operated steadily for 1 year with an NRR 1 of 0.8 kg N/m^3/day and hydraulic retention time of 24 hr. The dissolved oxygen (DO) was 0.1 to 0.4 mg/L, and the temperature was maintained at 32 ± 1°C. The values of the suspended solids (SS) and volatile suspended solids (VSS) of the inoculums were 7.36 g/L and 3.44 g/L, respectively. Synthetic wastewater was composed of NH_4HCO_3 as the ammonium (NH_4+) source, basic nutrients (10.0 mg/L NaH_2PO_4, 58.6 mg/L $MgSO_4 \cdot 7H_2O$, and 5.7 mg/L $CaCl_2 \cdot 2H_2O$), and trace elements (Graaf, 1996). In 1.0 L synthetic wastewater, 1.25 mL trace elements were supplemented. $KHCO_3$ solution

(1250 mg/L) was added to buffer the influent pH (8.0–8.5). Cubic sponges with reddish biofilms were threaded together and hung in the nitritation-anammox reactor at a fill rate of 12.5%. Flocculent sludge (2 L) and granular sludge (0.3 L) were also inoculated into the reactor. The initial biomass concentrations of the flocculent and granular sludge were 1.5 and 1.8 g MLSS/L, respectively. CTC (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in water to obtain a stock solution of 10 g/L. Then, a certain volume of CTC stock solution was added to the influent to investigate the effects of the antibiotics.

1.2. Continuous nitritation-anammox experiments

The nitritation-anammox reactor used in this study was an integrated fixed-biofilm and activated sludge reactor. As shown in Fig. 1, the working volume of the reactor was 20 L (100 cm × 10 cm × 50 cm). The system was divided into five equal zones by bafflers, and was aerated by a compressor with fine diffusers on the bottom of the system. The activated sludge settled in the cylindrical settling tank (8 L) and then was returned to the inlet of the system (recirculation flow rate of 1:1). The solid retention time was 20 days and the flocculent sludge was removed at a rate of 5% per day. A black cloth enclosure was used to shield the reactor from light to inhibit growth of the photosynthetic bacteria (Van, 1996). The temperature of the reactor was controlled at 32 ± 1°C and the DO concentration was maintained at 0.1–0.4 mg/L. The entire experiment lasted 140 days, which was divided into three operational phases according to the influent CTC concentration and experimental objectives, as described in Table 1. During phase I (days 1–70), the nitritation-anammox process was established in the combined reactor. The influent NH_4^+ concentration was gradually increased from 180 to 540 mg/L from Run 1 to Run 3. Meanwhile, the influent nitrogen loading rate (NLR) was increased from 0.36 to 0.87 kg N/m^3/day. In phase II (days 71–94), CTC was added to the influent to investigate the effects of the antibiotics. In Run 4 (days 71–84), the concentration of CTC was 60 mg/L. When the NRR was decreased, the influent CTC concentration decreased to 20 mg/L and the NLR decreased to 0.31–0.32 kg N/m^3/day in Run 5 (days 85–94). In phase III (days 95–140), the concentration of CTC was removed from the influent to recover the system performance. The NLRs in Run 6 (days 95–110) and Run 7 (days 111–120) were 0.30–0.35 and 0.18–0.20 kg N/(m^3·day), respectively. In Run 8 (days 121–140), anammox granules inoculants were added to the reactor to recover the nitrogen removal efficiency.

1.3. Short-term CTC inhibition

Batch tests were performed in 250 mL serum bottles to test the short-term effects of CTC on the nitritation-anammox process. The quantities of the biomass in each bottle were adjusted until the NRR of each reactor was 0.2 kg N/(m^3·day). CTC (Sigma) was dissolved in water to obtain a stock solution of 10,000 mg/L. Then CTC stock solution was added to the batch test bottles to achieve final concentrations of 0, 20, 50, 200, 400, and 800 mg/L. Three sets of runs were conducted to ensure the reproducibility of the results. The bottles were placed in a thermostatic shaker at 32 ± 1°C at 100 r/min. These
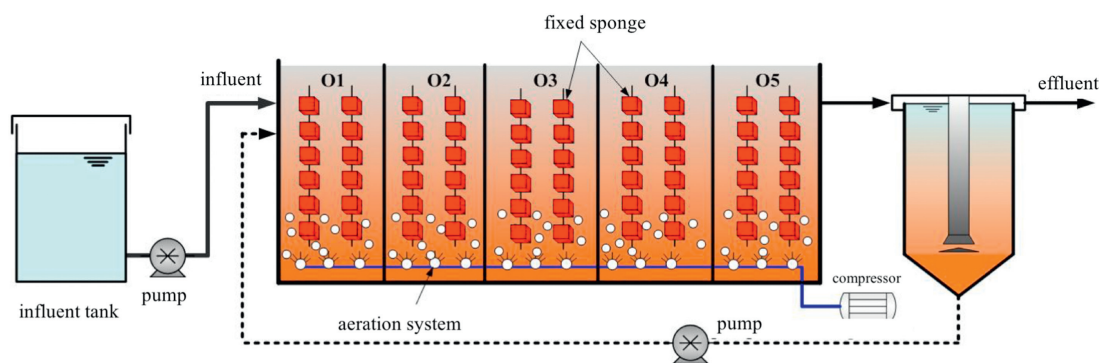
Fig. 1 – Scheme of nitritation-anammox reactor used in the experiment.

conditions were adopted to provide optimized temperature and limited oxygen for the nitritation-anammox reactor.

1.4. Chemical analytical methods

The samples were collected from the reactor and centrifuged at 3000 r/min for 2 min. The supernatants were filtered using 0.45 μm acetate cellulose membranes before analysis. Chemical oxygen demand, NH_4^+, nitrate (NO_3^-), nitrite (NO_2^-), mixed liquor suspend solid (MLSS), and VSS were measured according to standard methods (APHA, 2005). The total nitrogen (TN) level was measured using a Multi N/C 3100 Meter (Jena AG, Jena, Germany). Online monitors were also set as pilot- and full-scale reactors to measure the NH_4^+, NO_3^-, pH, temperature, and DO concentration (WTW Company, Weilheim, Germany).

2. Calculations

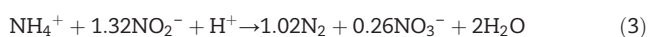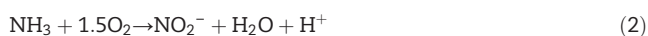### 2.1. The modified non-competitive inhibition model

The modified non-competitive inhibition model (Eq. (1)) was applied to evaluate the inhibitory characteristics of CTC on the nitritation-anammox process.

$$I = \left(1 - \frac{NRR_i}{NRR_0}\right) \times 100\% \qquad (1)$$

where, I is the inhibition response, NRR_i (hr) is the nitrogen removal rate at time i, and NRR_0 is the initial nitrogen removal rate.

2.2. Calculation of nitrogen transformation of AOB and AnAOB

AOB and AnAOB were the main functional microorganisms for nitrogen biotransformation in the reactors. Part of NH_4^+ is oxidized to nitrite by AOB (Eq. (2)). Then, the remaining NH_4^+ and NO_2^- are converted to nitrogen gas by AnAOB (Eq. (3)) (Miao et al., 2016). The NH_4^+ conversion rate (ACR) by AOB (AOB-ACR) (Eq. (4)) and AnAOB (AnAOB-ACR) (Eq. (5)) is estimated to explain the activities of AOB and AnAOB.

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H_2O + H^+ \qquad (2)$$

$$NH_4^+ + 1.32NO_2^- + H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \qquad (3)$$

$$AOB-ACR = \frac{NH_4^+-N(inf-eff) - \frac{NH_4^+-N(inf-eff) - NO_2^--N(eff-inf) - NO_3^--N(eff-inf)}{2.06}}{t \times 1000} \qquad (4)$$

$$AnAOB-ACR = \frac{NH_4^+-N(inf-eff) - NO_2^--N(eff-inf) - NO_3^--N(eff-inf)}{2.06 \times t \times 1000} \qquad (5)$$

The NH_4^+-N_{inf}, NH_4^+-N_{eff}, NO_2^--N_{inf}, NO_2^--N_{eff}, NO_3^--N_{inf}, and NO_3^--N_{eff} were the NH_4^+-N, NO_2^--N, and NO_3^--N concentrations in the influent and effluent during the long-term operation, respectively; t (day) is the hydraulic retention time. It was assumed that nitrogen assimilation due to heterotrophic growth was neglected in the calculations.

Table 1 – The operational conditions of the reactor during different phases.

Phase	Runs	Operation period (day)	Influent NH_4^+ (mg/L)	HRT (hr)	NLR (kg N/(m³·day))	Influent CTC (mg/L)
I	Run 1	1–7	179–278	12	0.36–0.57	0
I	Run 2	8–38	311–391	15	0.52–0.63	0
I	Run 3	39–70	416–540	15	0.66–0.87	0
II	Run 4	71–84	411–436	12	0.82–0.88	60
II	Run 5	85–94	413–456	32	0.31–0.32	20
III	Run 6	95–110	407–463	32	0.30–0.35	0
III	Run 7	111–120	179–195	24	0.18–0.20	0
III	Run 8	121–140	195–216	24	0.20–0.28	0

HRT: hydraulic retention time; NLR: nitrogen loading rate; CTC: chlortetracycline.

2.3. DNA extraction, PCR, and qPCR

Samples were collected for molecular biological analysis from the continuous system. For biofilm and granular sludge, 0.25 g of wet weight biomass was collected. For the flocculent sludge, 1 mL mixed liquor was centrifuged at 10000 r/min for 20 min and the supernatants were discarded. The settled biomass was used for DNA extraction and PCR amplification. The ammonia monooxygenase of AOB (amoA), and 16S rRNA gene of the anammox bacteria (amx) were amplified using the primer sets amoA-1f/amoA-2r and Amx368f/Amx820r, respectively (Appendix A Table S1). The settled biomass was used for DNA extraction and qPCR. Three genes targeting AOB, anammox bacteria, and the universal bacterial 16S rRNA for the total bacteria were quantified for all samples using the SYBR-Green Real-time PCR Master Mix (Liu et al., 2012). The amplification efficiencies were based on slopes between 93.4% and 96.5% (Appendix A Table S2). Based on the calibration curves, the abundance of AOB and AnAOB (copies/μL DNA) was calculated as follows:

$$\text{abundance of AOB and AnAOB (copies/}\mu\text{L DNA)}$$
$$= \frac{\text{DNA concentration (ng/}\mu\text{L)}}{\text{DNA molecular (g/mol)}} \times n \times 6.02 \times 10^{23} \times 10^{-9} \qquad (6)$$

where n represents the dilution factor of DNA when preparing the qPCR reaction, and then the abundance was normalized to the SS of the samples as below:

$$\text{abundance of AOB and AnAOB (copies/mg SS)}$$
$$= \frac{\text{abundance of AOB and AnAOB (copies/}\mu\text{L DNA)} \times 80\ \mu\text{L}}{1.5\ \text{mL} \times \text{SS (g/L)}}$$
$$\qquad (7)$$

The relative abundance of AOB and AnAOB was calculated according to the abundance of AOB and AnAOB:

$$\text{relative abundance of AOB and AnAOB (copies/mg SS)}$$
$$= \frac{\text{abundance of AOB and AnAOB (copies/mg SS)}}{\text{total abundance of AOB and AnAOB (copies/mg SS)}} \qquad (8)$$

3. Data analysis

All reactions were performed in triplicate, and the results are expressed as the mean ± standard deviation. Analysis of variance was used to test the significance of the results, and $p < 0.05$ was considered statistically significant. A statistical comparison among variables was performed using the t-test for a normally distributed dataset with SPSS Version 18.

4. Results and discussion

4.1. Start-up of the nitritation-anammox reactor

During the experimental period of phase I (days 1–70), the nitritation-anammox process was successfully established by inoculating the anammox biofilm and granular sludge. As shown in Fig. 2, the average influent NLR and NRR in Run 1 were 0.47 ± 0.09 and 0.18 ± 0.04 kg N/m³/day, respectively.

The effluent nitrate (67 mg/L) was higher than the value of the nitritation-anammox reaction, indicating a high activity of nitrite-oxidizing bacteria (NOB). In Run 2, to decrease the production of NO_3^--N, NH_4^+-N in influent was increased to 311–391 mg/L to enhance the inhibitive effects of free ammonia on NOB (Peng et al., 2008). Meanwhile, the NLR was kept at 0.58 ± 0.04 kg N/(m³·day) by increasing the hydraulic retention time (HRT) from 12 to 15 hr. During Run 2, the NRR was significantly increased to 0.30 ± 0.04 kg N/(m³·day) ($p = 0.00 < 0.05$). In Run 3, 800 mL anammox granules were introduced to the reactor. NRR was gradually increased by increasing the influent ammonium and aeration rate. At the end of Run 3, the NH_4^+-N in effluent was nearly below 5 mg/L, the NO_2^--N was 14–18 mg/L, and the NLR and NRR slightly increased to 0.75 ± 0.05 and 0.57 ± 0.12 kg N/(m³·day), respectively ($p = 0.85 > 0.05$). The average TN removal efficiency was 75.6% ± 12.4%. In addition, the AOB-ACR and AnAOB-ACR were 0.29 ± 0.09 and 0.23 ± 0.07 kg N/(m³·day), respectively. The lab-scale reactor presented a similar nitrogen removal performance to the pilot-scale reactor, indicating the successful establishment of the nitritation-anammox reaction.

4.2. CTC impact of the nitritation-anammox reactor

During phase 2 (days 71–94), CTC was added to the influent to investigate the effect of antibiotics on the nitritation-anammox process. In Run 4, the influent CTC concentration was 60 mg/L. After CTC was added to the reactor, the average NLR increased to 0.85 ± 0.02 kg N/m³/day, whereas the NRR of the reactor decreased from 0.57 ± 0.12 to 0.48 ± 0.10 kg N/m³/day. Subsequently, the concentrations of both NH_4^+-N and NO_2^--N rapidly increased in the effluent. The average RN removal efficiency decreased to 56.9% ± 12.6%. After the addition of CTC, NO_3^--N in the effluent hardly increased but the effluent concentration of NH_4^+-N significantly increased. Furthermore, the AOB-ACR increased to 0.37 ± 0.03 kg N/(m³·day), whereas the AnAOB-ACR did not significantly change and was maintained at 0.23 ± 0.05 kg N/(m³·day). These results indicate that AnAOB had lower sensitivity to CTC stress than AOB initially. However, after 10 days, a simultaneous increase of NH_4^+-N and NO_2^--N was observed. NO_2^--N accumulation indicated that CTC significantly inhibited AnAOB activity. The inhibition of AnAOB went through a latent period, which was previously reported. In the presence of 100 CTC mg/L, the activity of granular anammox biomass was constant for the first 7 days, and subsequently decreased to 75% of the control activity at the end of the experiment (Lotti et al., 2012b). Yang et al. (2013a) also reported that with 50 mg/L OTC stress, the anammox activity of the reactor did not vary during the initial 5 days but dropped suddenly by 4.5 kg N/m³/day afterwards (Yang et al., 2013b). In this study, AOB was mainly located in the flocculent sludge and the surface of the biofilm, which was more venerable to the influent variations. Comparatively, AnAOB grew inside the granular sludge and biofilm, which could resist CTC inhibition for a short period. After accumulation of antibiotics in the granular sludge and biofilm, AnAOB activity quickly decreased (Jin et al., 2012). The nitritation-anammox process under low stress of CTC was also investigated. In Run 5, influent CTC concentration was decreased to 20 mg/L. The influent NLR was decreased to 0.32 ± 0.00 kg N/(m³·day) by extending the HRT
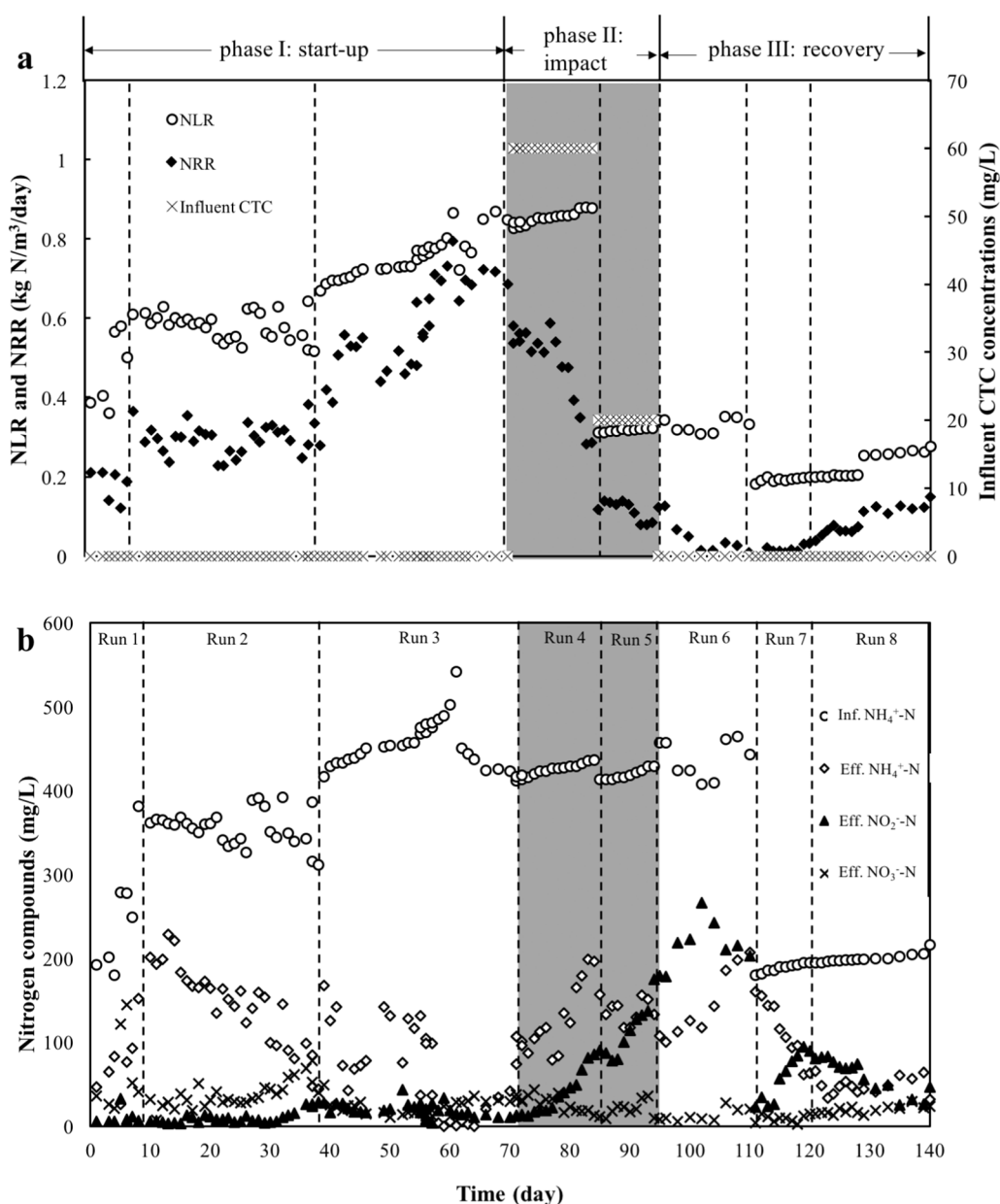
Fig. 2 – (a) The nitrogen removal performance of the nitritation-anammox system in different phases, and the variation of the nitrogen loading rate (NLR), nitrogen removal rate and influent chlortetracycline concentrations and (b) the variation of influent ammonium, effluent ammonium, nitrate and nitrite.

to 32 hr to recover the activity of AOB and AnAOB. However, the NRR significantly decreased to 0.11 ± 0.02 kg N/(m^3·day) (p = 0.00 < 0.05). The average total nitrogen removal efficiency still decreased to 36.1% ± 8.1%. The results suggested that nitritation-anammox reactor was vulnerable after inhibition of high CTC levels (60 mg/L). The reactor barely recovered after a long operational period even at a low CTC level. At the end of the Run 5, the AOB-ACR decreased to 0.16 ± 0.02 kg N/(m^3·day) and AnAOB-ACR decreased to 0.06 ± 0.01 kg N/(m^3·day). In this case, effluent NO_2^--N kept increasing to over 130 mg/L. The high concentration of NO_2^--N was unfavorable for the growth of AnAOB, which could be another reason for the slow recovery of nitritation-anammox reactor under low CTC concentrations.

4.3. Recovery of the nitritation-anammox reactor after CTC impact

In Run 6, CTC was totally evacuated from the influent to improve the reactor performance. However, no recovery was observed and the NRR decreased to 0.05 ± 0.05 kg N/(m^3·day) (p = 0.00 < 0.05). As shown in Fig. 2, when CTC was removed from the influent, AOB activity was gradually recovered, inducing a high NO_2^--N accumulation rate. The average NO_2^--N concentration was 210 mg/L in Run 6. The AOB-ACR increased to 0.20 ± 0.01 kg N/(m^3·day). However, AnAOB activity was severely inhibited, and the AnAOB-ACR decreased to 0.02 ±0.02 kg N/(m^3·day). In Run 7, the influent TN

concentration was further decreased to 200 mg/L to avoid the adverse effects of nitrite on AnAOB activity, and NLR was 0.19 ± 0.03 kg N/(m^3·day). However, the recovery of the reactor performance was so slow that the NRR was only 0.01 ± 0.05 kg N/(m^3·day) ($p = 0.00 < 0.05$). The AOB-ACR and AnAOB-ACR decreased to 0.07 ± 0.04 kg N/(m^3·day) and 0.01 ± 0.02 kg N/(m^3·day). In Run 8, anammox granular sludge of 800 mL (SS = 7.81 g/L and VSS = 3.67 g/L) was introduced into the reactor. The NRR was increased to 0.09 ± 0.03 kg N/(m^3·day) ($p = 0.601 > 0.05$). According to the experimental results, the decrease in influent CTC may cause severe NO$_2^-$-N accumulation and further inhibit AnAOB activity. The anammox sludge addition may improve the system conditions in the reactor by consuming excess NO$_2^-$-N, which promoted the bacteria community in the reactor. Similarly, in Yang's research, the recovery of nitrogen removal performance after oxytetracycline inhibition was accelerated by adding a biocatalyst (Yang et al., 2013b). AnAOB was reported to grow slowly and was very sensitive to the surrounding environment (Strous et al., 1998b). Moreover, CTC may be adsorbed, precipitated, or accumulated in the sludge, thereby leading to continuous inhibition of AnAOB. Therefore, recovery of the system performance requires a very long period, even upon biocatalyst addition.
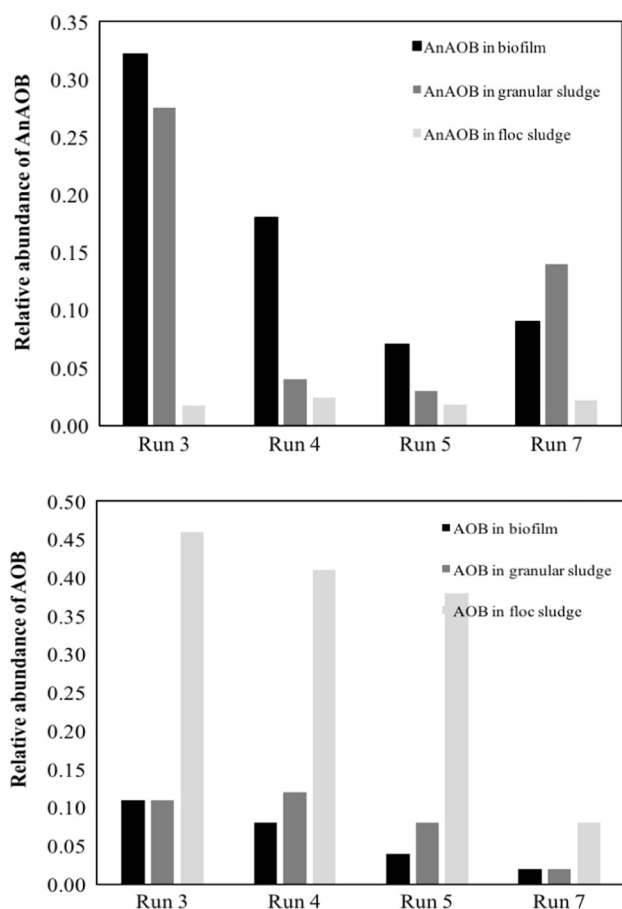

Fig. 3 – Relative abundance of anaerobic ammonium oxidizing bacteria (AnAOB) and ammonium oxidizing bacteria (AOB) in different runs.

4.4. Relative abundance of AOB and AnAOB

The relative abundance of AOB and AnAOB was studied at the end of each operation period (Fig. 3). During the stable period before CTC inhibition (Runs 1–3), the relative abundance of AnAOB in the biofilm and granular sludge (0.32 and 0.28, respectively) was much higher than that in the floc sludge (0.02) at the end of Run 3, whereas the highest relative abundance of AOB was found in the floc sludge (0.46). This indicated that AnAOB preferred the niche in biofilm and granular sludge, while AOB preferred the floc sludge. It was presumed that the inside of the biofilm and granular sludge was anaerobic in which AnAOB preferred to exist, whereas floc sludge provided enough DO for the growth of AOB. At the end of Run 4, the relative abundance of the AnAOB decreased from 0.60 to 0.22 when the nitrogen performance was inhibited. AnAOB in the biofilm decreased from 0.32 to 0.0.18, while it significantly decreased in the granular sludge from 0.28 to 0.04. These results suggest that biofilm can lessen the inhibitory characteristics of CTC on AnAOB better than granular sludge. The relative abundance of AOB in the floc sludge slightly decreased from 0.46 to 0.41, indicating that AOB bacteria was more tolerant to CTC inhibition than AnAOB. The CTC in the influent decreased to 20 mg/L (Run 5), the relative abundance of AnAOB continued to decrease to 0.10, and that of AOB decreased to 0.38. The results showed that the inhibition of CTC on AnAOB is irreversible. After the anammox granules were inoculated in the reactor, the relative abundance of the AnAOB in the biofilm increased from 0.07 to 0.09 and increased from 0.03 to 0.14. The results implied that the AnAOB mainly existed in the granules, and AnAOB was attached to the biofilm. The relative abundance of AOB in the floc sludge decreased from 0.38 to 0.08. In general, the total relative abundance of AOB and AnAOB was 0.12 and 0.25, respectively, in the reactor, indicating a balance between the amounts of these two microbes. The quantification of the functional bacteria corresponded well with the operation performance, suggesting that the molecular biological methods can be efficiently used to better understand the inhibitory effects of adverse factors on anammox processes. The results are
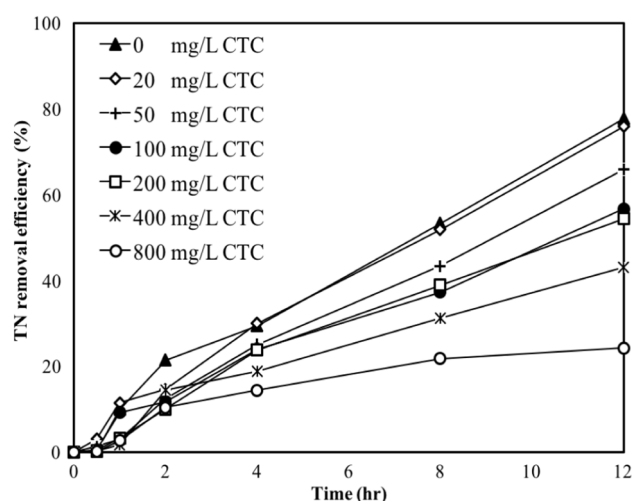

Fig. 4 – Total removal efficiency under different initial chlortetracycline concentrations.

Table 2 – Reported short-term antibiotics inhibition on anammox bacteria.

Antibiotic	Concentration (mg/L)	Exposure time (hr)	Activity inhibition	IC$_{50}$ (mg/L)	Reference
Chloramphenicol	250–1000	6	40%–80%	–	(Fernandez et al., 2009)
Tetracycline	100–1000	6	30%–80%	–	(Fernandez et al., 2009)
Oxytetracycline	100–1000	30	0%–50%	1000	(Lotti et al., 2012)
Sulfathiazole	100–1000	30	0%–70%	650	(Lotti et al., 2012)
Oxytetracycline	25–1100	–	–	517.5	(Yang et al., 2013b)
Oxytetracycline	25–100	7	55%–70%	–	(Noophan et al., 2012)
Chlorotetracycline	20–800	12		278.91	This study

–: unknown.

consistent with the previous studies, as the existence of genes coding for quorum sensing (QS) compounds was shown by Strous et al. (2006) In addition, Tang et al. (2015) demonstrated that the existence of the QS system among AnAOB could improve its growth rate.

4.5. Inhibitory effects of CTC on nitritation-anammox process

At a substrate concentration of 180 mg/L TN, the nitrogen removal activity of nitritation-anammox sludge was measured at different time points at CTC concentrations of 0, 20, 50, 200, 400, 800 mg/L (Fig. 4). When the concentration was below 200 mg/L, there was no obvious inhibitory effect. At levels higher than 400 mg/L, the inactivation time was approximately 4 hr. These results demonstrate that the TN removal rate was quickly inhibited at high CTC concentrations.

According to the modified non-competitive inhibition model (Eq. (1)), the IC$_{50}$ of CTC on TN removal performance was 498.24, 324.47, and 278.91 mg/L at exposure times of 4, 8, and 12 hr, respectively. Table 2 shows the other antibiotics that had inhibitory effects toward AnAOB. There might be several reasons for this discrepancy. First, various antibiotics have their own inhibitory mechanisms toward CTC. Second, the different reactor and different AnAOB, in our study, it is the single-stage Anammox reactor and the bacteria mainly include AnAOB and AOB.

5. Conclusions

In summary, this study provides the IC$_{50}$ of CTC on TN removal performance, which was 278.91 mg/L in a short period (12 hr). The observation from continuous experiments reveals that the inhibition by 60 mg/L CTC of the nitritation-anammox process is irreversible, and AnAOB has lower sensitivity to CTC stress than AOB at the beginning of CTC addition. The qPCR data indicated that AnAOB preferred the niche in biofilm and granular sludge, whereas AOB preferred the floc sludge. In addition, adding anammox sludge was an efficient strategy for performance recovery of the nitritation-anammox process.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2017.11.005.

REFERENCES

Alvarez, J.A., Otero, L., Lema, J.M., Omil, F., 2010. The effect and fate of antibiotics during the anaerobic digestion of pig manure. Bioresour. Technol. 101, 8581–8586.

Baquero, F., Martinez, J.L., Canton, R., 2008. Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 19, 260–265.

Fernandez, I., Mosquera-Corral, A., Campos, J.L., Mendez, R., 2009. Operation of an Anammox SBR in the presence of two broad-spectrum antibiotics. Process Biochem. 44, 494–498.

Graaf, A.A.V.D., 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142, 2187–2196.

Hou, J., Wang, C., Mao, D.Q., Luo, Y., 2016. The occurrence and fate of tetracyclines in two pharmaceutical wastewater treatment plants of northern China. Environ. Sci. Pollut. R. 23, 1722–1731.

Jin, R.C., Yang, G.F., Yu, J.J., Zheng, P., 2012. The inhibition of the Anammox process: a review. Chem. Eng. J. 197, 67–79.

Jin, R.C., Yang, G.F., Zhang, Q.Q., Ma, C., Yu, J.J., Xing, B.S., 2013. The effect of sulfide inhibition on the ANAMMOX process. Water Res. 47, 1459–1469.

Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H., van Loosdrecht, M.C., 2014. Full-scale partial nitritation/anammox experiences—an application survey. Water Res. 55, 292–303.

Liu, S.T., Horn, H., 2012. Effects of Fe(II) and Fe(III) on the single-stage deammonification process treating high-strength reject water from sludge dewatering. Bioresour. Technol. 114, 12–19.

Liu, M.M., Zhang, Y., Yang, M., Tian, Z., Ren, L.R., Zhang, S.J., 2012. Abundance and distribution of tetracycline resistance genes and mobile elements in an oxytetracycline production wastewater treatment system. Environ. Sci. Technol. 46, 7551–7557.

Liu, B., Li, Y.X., Zhang, X.L., Wang, J., Gao, M., 2015. Effects of chlortetracycline on soil microbial communities: comparisons of enzyme activities to the functional diversity via Biolog EcoPlates (TM). Eur. J. Soil Biol. 68, 69–76.

Loosdrecht, M.C.M.V., Damir, B., 2014. Water treatment. Anticipating the next century of wastewater treatment. Science 344, 1452–1453.

Lotti, T., Cordola, M., Kleerebezem, R., Caffaz, S., Lubello, C., van Loosdrecht, M.C.M., 2012a. Inhibition effect of swine wastewater heavy metals and antibiotics on anammox activity. Water Sci. Technol. 66, 1519–1526.

Lotti, T., Cordola, M., Kleerebezem, R., Caffaz, S., Lubello, C., van Loosdrecht, M., 2012b. Inhibition effect of swine wastewater heavy metals and antibiotics on anammox activity. Water Sci. Technol. 66, 1519–1526.

Miao, Y.Y., Zhang, L., Yang, Y.D., Peng, Y.Z., Li, B.K., Wang, S.Y., et al., 2016. Start-up of single-stage partial nitrification-anammox process treating low-strength swage and its restoration from nitrate accumulation. Bioresour. Technol. 218, 771–779.

Nhat, P.T., Biec, H.N., Mai, N.T.T., Thanh, B.X., Dan, N.P., 2014. Application of a partial nitration and anammox system for the old landfill leachate treatment. Int. Biodeter. Biodegr. 95, 144–150.

Peng, Y.Z., Zhang, S.J., Zeng, W., Zheng, S.W., Mino, T., Satoh, H., 2008. Organic removal by denitritation and methanogenesis and nitrogen removal by nitritation from landfill leachate. Water Res. 42, 883–892.

Stone, J.J., Dreis, E.K., Lupo, C.D., Clay, S.A., 2011. Land application of tylosin and chlortetracycline swine manure: impacts to soil nutrients and soil microbial community structure. J. Environ. Sci. Health., Part B 46, 752–762.

Strous, M., Heijnen, J.J., Kuenen, J.G., Jetten, M.S.M., 1998a. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl. Microbiol. Biotechnol. 50, 589–596.

Strous, M., Heijnen, J.J., Kuenen, J.G., Jetten, M.S.M., 1998b. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl. Microbiol. Biotechnol. 50, 589–596.

Strous, M., Pelletier, E., Mangenot, S., Rattei, T., Lehner, A., Taylor, M.W., et al., 2006. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440, 790–794.

Tang, C.J., Zheng, P., Chen, T.T., Zhang, J.Q., Mahmood, Q., Ding, S.A., et al., 2011. Enhanced nitrogen removal from pharmaceutical wastewater using SBA-ANAMMOX process. Water Res. 45, 201–210.

Tang, X., Liu, S.T., Zhang, Z.T., Zhuang, G.Q., 2015. Identification of the release and effects of AHLs in anammox culture for bacteria communication. Chem. Eng. J. 273, 184–191.

Tong, L., Huang, S.B., Wang, Y.X., Liu, H., Li, M.J., 2014. Occurrence of antibiotics in the aquatic environment of Jianghan Plain, central China. Sci. Total Environ. 497, 180–187.

Van, d.G.A.A., 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiol., (UK) 142, 2187–2196.

van der Star, W.R.L., Abma, W.R., Blommers, D., Mulder, J.W., Tokutomi, T., Strous, M., et al., 2007. Startup of reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam. Water Res. 41, 4149–4163.

Wang, C., Zhao, Y.C., Xie, B., Peng, Q., Hassan, M., Wang, X.Y., 2014. Nitrogen removal pathway of anaerobic ammonium oxidation in on-site aged refuse bioreactor. Bioresour. Technol. 159, 266–271.

Yang, G.F., Zhang, Q.Q., Jin, R.C., 2013a. Changes in the nitrogen removal performance and the properties of granular sludge in an Anammox system under oxytetracycline (OTC) stress. Bioresour. Technol. 129, 65–71.

Yang, G.F., Zhang, Q.Q., Jin, R.C., 2013b. Changes in the nitrogen removal performance and the properties of granular sludge in an Anammox system under oxytetracycline (OTC) stress. Bioresour. Technol. 129, 65–71.

Zhang, Z.Z., Zhang, Q.Q., Guo, Q., Chen, Q.Q., Jiang, X.Y., Jin, R.C., 2015. Anaerobic ammonium-oxidizing bacteria gain antibiotic resistance during long-term acclimatization. Bioresour. Technol. 192, 756–764.

Zielezny, Y., Groeneweg, J., Vereecken, H., Tappe, W., 2006. Impact of sulfadiazine and chlorotetracycline on soil bacterial community structure and respiratory activity. Soil Biol. Biochem. 38, 2372–2380.