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Q1 Inhibitive effects of chlortetracycline on performance of the nitritation-anaerobic ammonium oxidation (anammox) process and strategies for recovery

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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.

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

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(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 $N/(m^3{\cdot}day)$, 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 $N/(m^3{\cdot}day)$. 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

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