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# Changes in the nitrogen removal performance and the properties of granular sludge in an Anammox system under oxytetracycline (OTC) stress

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## HIGHLIGHTS

- The short- and long-term effects of oxytetracycline (OTC) on Anammox.
- The changes in properties of granular sludge after OTC stress.
- The recovery of Anammox activity and performance after OTC inhibition.

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## ABSTRACT

The short- and long-term effects of oxytetracycline (OTC) on the anaerobic ammonium oxidation (Anammox) process were evaluated. The OTC inhibition of Anammox was substrate-, and especially nitrite-, dependent. The $IC_{50}$ of OTC in the batch tests on an Anammox mixed culture was calculated to be 517.5 mg $L^{-1}$. The long-term effects of OTC on the Anammox process were examined in a continuous-flow upflow anaerobic sludge blanket reactor. Fifty milligrams per liter of OTC significantly decreased the nitrogen removal rate from 12.4 to 2 kg N $m^{-3}$ $d^{-1}$ within 26 days. The recovery of Anammox performance after OTC inhibition was accelerated by adding biocatalyst. In contrast to the modified Stover–Kincannon model, the modified Boltzmann model accurately simulated the recovery of Anammox performance. OTC presented in the influent led to sludge hardening and cell lysis. A poor settling property of Anammox sludge was also observed.



## 1. Introduction

Nitrogen pollution has become an increasingly important issue, and several novel and promising nitrogen removal processes have been developed (Sabumon, 2007). Anaerobic ammonium oxidation (Anammox), first discovered in a denitrifying fluidized bed reactor in 1995 (Mulder et al., 1995), is one of these new processes. Under anaerobic conditions, Anammox bacterium oxidizes ammonium using nitrite as electron acceptor to produce dinitrogen gas (Strous et al., 1999a). As an economical and sustainable technology for biological nitrogen removal, Anammox has been extensively studied (van Hulle et al., 2010; Jin et al., 2012). Antibiotics are administered extensively to humans, animals and aquaculture systems as therapeutics (Kümmerer, 2009). High antibiotic concentrations have been detected in aquatic environments, including bank filtrates, surface water and even ground water (Kümmerer, 2009). Convincing evidence has shown that Anammox activity is inhibited by antibiotics (van de Graaf et al., 1995; Fernández et al., 2009; Tang et al., 2011a).

Fernández et al. (2009) reported that, at a final concentration of 100 mg $L^{-1}$, tetracycline hydrochloride (TCH), which belongs to a class of compounds known as the tetracyclines, seriously suppressed Anammox activity in a short-term test. The long-term effect of TCH on Anammox was conducted in a sequencing batch granular sludge reactor with a nitrogen loading rate (NLR) of 0.3 kg N $m^{-3}$ $d^{-1}$. A TCH concentration of 10 mg $L^{-1}$ decreased the specific Anammox activity (SAA) of the sludge by 60%, and nitrite accumulated at a TCH level of 50 mg $L^{-1}$. Like TCH, oxytetracycline (OTC) is a member of the tetracyclines. Prior to the present study, the effect of OTC on Anammox was unknown. OTC, a common antibiotic with a broad range of activity and a low cost (Álvarez et al., 2010), can be found in some nitrogen-rich wastewaters, such as OTC production wastewater and swine wastewater digester liquor. It was reported that the OTC level is above 50 mg $L^{-1}$ in the outflow of an OTC production facility in China (Li et al., 2008), and the OTC concentration in surface water is up to 0.34 mg $L^{-1}$ (Kümmerer, 2009).

It has been reported that OTC suppressed the bioactivity of various microorganism (Kümmerer, 2009; Álvarez et al., 2010). To the

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