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Susceptibility, resistance and resilience of anammox biomass to nanoscale copper stress

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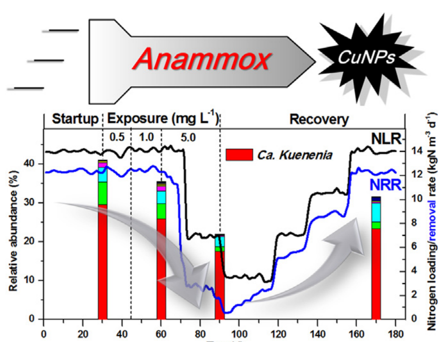
HIGHLIGHTS

- 5.0 mg L^{-1} CuNPs deprived the nitrogen removal capacity of the reactor in 30 days.
- The abundance of "*Candidatus Kuenenia*" decreased greatly with increased CuNPs.
- Copper resistance genes associated with the Cus, Cop and Pco systems were enriched.
- The anammox performance could recover completely after withdrawing CuNPs.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing use of engineered nanoparticles (NPs) poses an emerging challenge to biological wastewater treatment. The long-term impact of CuNPs on anaerobic ammonium oxidation (anammox) process was firstly investigated in this study. The nitrogen removal capacity of anammox reactor was nearly deprived within 30 days under the stress of 5.0 mg L^{-1} CuNPs and the relative abundance of anammox bacteria (Ca. *Kuenenia*) was decreased from 29.59% to 17.53%. Meanwhile, copper resistance genes associated with the Cus, Cop and Pco systems were enriched to eliminate excess intracellular copper. After the withdrawal of CuNPs from the influent, the nitrogen removal capacity of anammox biomass recovered completely within 70 days. Overall, anammox biomass showed susceptibility, resistance and resilience to the stress of CuNPs. Therefore, the potential impacts of ENPs on anammox-based processes should be of great concern.

1. Introduction

Nanotechnology has made a spurt of progress since the end of the 20th century. Currently, engineered nanoparticles (NPs) have been widely applied in many consumer products and industrial fields (Wang et al., 2016a). For example, CuNPs are of great value in fuel cells, microelectronics, and bioactive coatings because of their thermal and electrical conductivity properties and in textiles, skin products, inks and wood preservation products because of their bactericidal properties (Chen et al., 2012). However, in the processes of production, storage, use, and disposal of NP-containing products, the release of NPs to the environment inevitably occurs (Gottschalk and Nowack, 2011). Accordingly, the environmental risk of NPs has become a rising public concern

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and a hotspot of research. Wastewater treatment plants (WWTPs) have been reported as one of the major acceptors of released NPs at the end of their lifetime (Musee et al., 2011; Yang et al., 2013); therefore, the potential effects of NPs on the function of biological wastewater treatment have been investigated extensively, including metallic (Cu, Ag, Fe) and metal oxide (CuO, ZnO, Fe_3O_4, TiO_2, CeO_2, Al_2O_3) NPs (Wang and Chen, 2016; Yang et al., 2013; Yuan et al., 2015). As for CuNPs, previous studies reported that 10 mg L^{-1} CuNPs in wastewater did not have an obvious acute toxic effect on ammonia oxidizing bacteria (Ganesh et al., 2010), whereas the direct addition of 17.4 mg L^{-1} CuNPs to an anaerobic fermentation reactor inhibited both hydrolysis and acidification, thereby reducing the volatile fatty acid (VFA) production (Chen et al., 2014). In addition, the number of denitrifiers in the sequencing batch reactor was higher after long-term exposure to 5 mg L^{-1} CuNPs (Chen et al., 2012). Overall, the impacts of CuNPs on bacterial function differ from species to species.

Anaerobic ammonium oxidation (anammox) process, in which anammox bacteria directly oxidize ammonium to dinitrogen gas using nitrite as the electron acceptor in the absence of oxygen and organic carbon, is one of the most important recent discoveries in the microbial nitrogen cycle (Hu et al., 2011). The distribution of anammox bacteria has been shown to be ubiquitous in various natural habitats, such as marine ecosystems, inland lakes and agricultural soil, where it accounts for 9–40%, 4–37% and 50% of the nitrogen loss, respectively (Hu et al., 2011). Since the discovery of anammox in 1995, several different processes based on anammox bacteria have been developed to achieve highly efficient and autotrophic nitrogen removal from wastewater (Lackner et al., 2014). The integration of anammox process into WWTPs can improve the energy efficiency of treatment facilities (Kuenen et al., 2011); thus, anammox-based processes have been widely regarded as an innovative and sustainable alternative to the classical activated sludge process, i.e., nitrification-denitrification (Lackner et al., 2014; Zhang et al., 2016a). Globally, the amount of full-scale anammox installations had exceeded 100 by early 2015 (Lackner et al., 2014). Moreover, an increasing number of programs to upgrade and renovate WWTPs are adopting anammox-based processes (Lackner et al., 2014; Morales et al., 2015).

From a long-term perspective, NPs pose a potential challenge to the emerging anammox process. However, to the best of our knowledge, whether the presence of NPs in the wastewater causes negative effects on anammox bacteria remains unclear. The investigation of CuNPs on other bacteria cannot reflect their impact on anammox bacteria (Chen et al., 2014, 2012; Ganesh et al., 2010). Our preliminary study showed that CuNPs made a difference with CuONPs, AgNPs and ZnONPs in the acute toxicity to anammox biomass (Zhang et al., 2017a,b). Therefore, in this study, CuNPs were selected as model NPs to investigate their long-term impacts on the granule-based anammox process. First, the nitrogen removal performance of the anammox reactor was evaluated under an increased stress of CuNPs (0.5 mg L^{-1} for 15 days, 1.0 mg L^{-1} for 15 days, and 5 mg L^{-1} for 30 days) and during the subsequent recovery phase. In addition, the dynamics of the microbial community were also tracked by the high-throughput sequencing technique. Finally, the relationship between the operational parameters and the microbial characteristics was also assessed by a redundancy analysis (RDA) and a Pearson correlation analysis.

2. Materials and methods

2.1. Anammox reactor and seeding sludge

A continuous-flow experiment was conducted in an up-flow anaerobic sludge blanket (UASB) reactor, which was fabricated from Plexiglas with an internal diameter of 60 mm and an effective volume of 1.0 L. Anammox seeding sludge was harvested from a laboratory-scale UASB reactor (3.0 L), which had been operating stably in a temperature-controlled (35 ± 1 °C) room for more than one year. The specific anammox activity (SAA), the mean diameter and the extracellular polymeric substance (EPS) content of these mature anammox granules were 522.3 ± 41.5 mgTN g^{-1} volatile suspended solids (VSS) d^{-1}, 2.24 ± 1.4 mm and 276.5 ± 9.9 mg g^{-1} VSS, respectively.

2.2. Synthetic wastewater and source of CuNPs

Synthetic wastewater containing substrates, minerals and trace elements was pumped into the reactor. Ammonium and nitrite were supplied as substrates as needed in the forms of $(NH_4)_2SO_4$ and $NaNO_2$, respectively. The mineral medium was composed of 840 mg L^{-1} $NaHCO_3$, 300 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 5.6 mg L^{-1} $CaCl_2{\cdot}2H_2O$ and 10 mg L^{-1} NaH_2PO_4. Trace element solution I consisted of 5 g L^{-1} EDTA and 9.14 g L^{-1} $FeSO_4{\cdot}7H_2O$, and trace element solution II was composed of 15 g L^{-1} EDTA, 0.014 g L^{-1} H_3BO_4, 0.25 g L^{-1} $CuSO_4{\cdot}5H_2O$, 0.22 g L^{-1} $NaMoO_4{\cdot}2H_2O$, 0.43 g L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.21 g L^{-1} $NiCl_2{\cdot}6H_2O$, 0.99 g L^{-1} $MnCl_2{\cdot}4H_2O$ and 0.24 g L^{-1} $CoCl_2{\cdot}6H_2O$. Trace element solutions I and II were added at 1.25 mL per liter of wastewater.

Commercially produced CuNPs (10–30 nm) of 99.9% purity were purchased from the Aladdin Reagent Co. Ltd., China. A stock suspension of CuNPs (2 g L^{-1}, pH 7.5) was prepared according to previously described methods (Mu et al., 2011). To enhance the stability of the stock suspension, 0.1 mM sodium dodecylbenzene sulfonate (SDBS) was added as a dispersing reagent and the stock suspension was subsequently sonicated for 1 h in an ultrasonic bath (25 °C, 40 kHz, 250 W) to break up the aggregates. The stock suspension of CuNPs was prepared for each use and immediately added to the reactor influent to reach the desired exposure concentration.

2.3. Experiment setup and operational strategy

The reactor was inoculated with 0.5 L of settled anammox granules on day 0, and the initial sludge concentration was approximately 16.3 gVSS L^{-1}. The reactor was covered completely with black cloth to avoid the growth of phototrophic microorganisms and then placed in a dark and thermostatic room at 35 ± 1 °C. To obtain a high volumetric nitrogen removal rate (NRR), the reactor was operated at a relatively low influent substrate level of 280 mgN L^{-1} and high hydraulic retention time (HRT) of 0.96 h. The sludge in the reactor was mainly lost by sampling (100 mL per month) and washout (~20 mgSS L^{-1}); thus, the sludge retention time (SRT) was calculated as approximately 36.8 d. To avoid the inhibition caused by nitrite or free ammonia, ammonium was supplied in excess with an equimolar amount of nitrite, and the influent pH was maintained at approximately 7.8. The dissolved oxygen concentration in the reactor was below the detection limit.

The estimated amount of NPs in the wastewater ranged from μg L^{-1} to mg L^{-1} (Musee et al., 2011), and previous studies have mainly investigated the impact of NPs at a range of 1–100 mg L^{-1} or mg g^{-1} suspended solids (SS) (Mu et al., 2012; Musee et al., 2011; Walden and Zhang, 2016; Zheng et al., 2011). Accordingly, in this study, the effects of environmentally relevant levels of CuNPs on anammox sludge were investigated. To reach a comprehensive conclusion, the potential effects of higher CuNP loads were also considered because of their wider large-scale production (Chen et al., 2012; Mu et al., 2012). Thus, 0.5 mg L^{-1} CuNPs was added into the influent on Day 31, and then the impact of 1.0 and 5.0 mg L^{-1} CuNPs on the anammox process was tested. Based on the initial sludge concentrations, the corresponding specific