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Transient disturbance of engineered ZnO nanoparticles enhances the resistance and resilience of anammox process in wastewater treatment

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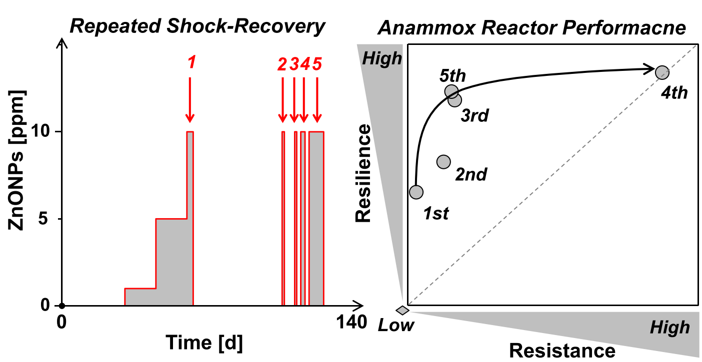
HIGHLIGHTS

- The presence of 1–5 mg L^{-1} ZnONPs did not affect anammox reactor performance.
- 10 mg L^{-1} ZnONP shock deprived 90% of the nitrogen removal capacity within 3 days.
- The resistance and resilience was enhanced by intermittent perturbations.
- The functional specificity of the anammox community was improved by repeated training.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing use of engineered nanoparticles (NPs) in consumer and industrial products raises concerns about their environmental impacts, but their potential influence on anaerobic ammonium oxidation (anammox) process in wastewater treatment remains unknown. In this study, the response of granule-based anammox reactor to different loads of ZnONPs was investigated. The introduction of 1–5 mg L^{-1} ZnONPs did not affect reactor performance, but 90% of the nitrogen removal capacity was deprived by a shock of 10 mg L^{-1} ZnONPs within 3 days. Anammox activity was significantly inhibited, but no significant stimulation of intracellular reactive oxygen species (ROS) production or extracellular lactate dehydrogenase (LDH) activity was observed. The inhibition was thus mainly due to the accumulation of toxic Zn(II) ions in anammox biomass. However, the resistance and resilience of this anammox reactor to ZnONPs were enhanced by intermittent perturbations in the mode of "shock-recovery". The up-regulated abundance of Zn(II)-exporter ZntA might contribute to the enhanced resistance. In addition, these repeated transient disturbances improved the functional specificity of the anammox community despite the reduction of its diversity. Overall, these results may provide useful references for evaluating and controlling the risk of NPs to anammox process.

1. Introduction

Engineered nanoparticles (NPs) are widely and increasingly produced for use in many commercial industrial and consumer products due to their unique properties and are becoming more frequently consumed in these products (Mu et al., 2012; Mu and Chen, 2011; Yang

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et al., 2013). Particularly, ZnONPs have been widely adopted for use in antibactericidal coatings, catalysts, biomedicine, skin creams, and toothpastes because of their magnetic, electrical, and optical properties (Mu et al., 2012). The increased production and widespread use of NP-containing products inevitably causes the release of NPs to the environment during their production, usage or disposal (Wang and Chen, 2016). Wastewater treatment plants (WWTPs) have been considered to be one of the major sinks of the released NPs at the end of their lifetime (Wang and Chen, 2016; Yang et al., 2013). The estimated amounts of NPs in wastewater range from μg L^{-1} to mg L^{-1}; however, their high affinity for sludge may induce the accumulation of NPs (Brar et al., 2010; Zhang et al., 2017a). Although the current level of NPs in real wastewater is relatively low, their concentrations will probably increase in view of their large-scale production and application (Wang and Chen, 2016). Therefore, the potential risk of NPs to biological wastewater treatment has become a rising public concern as well as a hotspot of research.

Recently, the impacts of NPs on biological wastewater treatment have been reported extensively, and they influence nitrification, denitrification, anaerobic digestion and enhanced biological phosphorus removal (Gu et al., 2014; Mu et al., 2011, 2012; Yang et al., 2013; Zheng et al., 2011). However, limited information is available regarding the impacts of NPs on anaerobic ammonium oxidation (anammox) process (Gonzalez-Estrella et al., 2017; Zhang et al., 2017a, 2017b, 2017c). Anammox process, in which ammonium is directly converted to dinitrogen gas by anammox bacteria that use nitrite as an electron acceptor but have no need of oxygen and organic carbon, has been widely regarded as an innovative and sustainable alternative to conventional biological nitrogen removal (BNR) technologies (Kartal et al., 2010; Zhang et al., 2015). According to the latest survey, the amount of full-scale anammox installations worldwide exceeded 100 by early 2015 (Lackner et al., 2014). In total, 75% of these installations are used to treat sidestream (reject water), and anammox is also a promising process for mainstream treatment, based on recent advances (Laureni et al., 2016; Lotti et al., 2014; Yeshi et al., 2016).

From a long-term perspective, the wide use of NPs poses an emerging challenge to the application of anammox process. ZnONPs are frequently selected as a model to investigate, because ZnONPs have been confirmed to be present in sewage sludge and effluents (Zheng et al., 2011). Previous studies reported that the presence of 10 mg L^{-1} ZnONPs significantly inhibited the growth of *Bacillus subtilis* and *Escherichia coli* (Adams et al., 2006). Another report showed that 10–50 mg L^{-1} ZnONPs substantially inhibits the gene expressions and catalytic activities of key denitrifying enzymes, leading to great increases in N_2O emission and decreases in nitrate removal (Zheng et al., 2014). In addition, 10–50 mg L^{-1} of ZnONPs could reduce the nitrogen and phosphorus removal efficiencies of activated sludge (Zheng et al., 2011). However, the methane production of anaerobic granular sludge was not significantly inhibited by 10–50 mg L^{-1} ZnONPs (Mu et al., 2012). These studies, taken as a whole, indicated that the adverse effect of ZnONPs depended on the species of bacteria being used and thus could not reflect their impact on anammox bacteria. Previous studies reported the toxic effects of ZnONPs on completely autotrophic nitrogen removal over nitrite (CANON) process (Zhang et al., 2017, 2018). However, to the best of our knowledge, the impact of ZnONPs on anammox process has not been well documented. Our preliminary study indicated that unlike CuNPs, ZnONPs showed no acute toxicity to anammox granules (Zhang et al., 2017c). However, the actual effect of NPs on highly aggregated biomass may be not visible during several hours of exposure and requires long-term observations to verify (Mu et al., 2012; Zhang et al., 2017b, 2017c).

Therefore, in this study, the chronic response of anammox granules to ZnONP exposure was investigated in terms of the reactor performance as well as the community dynamics. More particularly, the resistance and resilience of anammox process to the shock of ZnONPs were quantitatively evaluated. Interestingly, the magic role of repeated transient disturbances to the anammox reactor was also unraveled.

2. Materials and methods

2.1. Origin of anammox biomass and nanoparticles

Commercially produced ZnONPs (30 ± 10 nm) of 99.9% purity were purchased from Aladdin Reagent Co. Ltd., China. The X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectra of these ZnONPs are shown in Figs. S1 and S2. The stock suspension (2 g L^{-1}, pH 7.5) was prepared according to previously described methods (Mu et al., 2012; Mu and Chen, 2011). ZnONPs were added to distilled water containing 0.1 mM sodium dodecylbenzene sulfonate (SDBS), a dispersing reagent, to enhance the stability of the stock suspension. Before each use, the stock suspension was sonicated for 1 h in an ultrasonic bath (25 °C, 40 kHz, 250 W) to break aggregates. Dynamic light scattering (DLS) analysis using a Malvern Zetasizer NanoZS (Malvern Instruments, UK) indicated that the average particle size of the ZnONPs in the stock suspension was approximately 94 nm.

Mature anammox granules were harvested from a laboratory-scale up-flow anaerobic sludge blanket (UASB) reactor that had been operating stably for more than one year under thermostatic (35 ± 1 °C) conditions. These anammox granules possess a mean diameter of 2.24 ± 1.4 mm, an extracellular polymeric substance (EPS) content of 276.5 ± 17.9 mg g^{-1} volatile suspended solids (VSS), and a specific anammox activity (SAA) level of 522.3 ± 41.5 mgTN g^{-1}VSS d^{-1}.

2.2. Reactor setup and operation

A UASB reactor fabricated from Plexiglas, with an internal diameter of 6 cm and a working volume of 1.0 L, was used for continuous-flow experiments. This reactor was inoculated with 1.0 L of mature anammox granules to achieve full capacity, and the initial biomass concentration was approximately 20.1 gVSS L^{-1}; this reactor was then placed in a dark and thermostatic room at 35 ± 1 °C. Synthetic wastewater containing substrates, minerals and trace elements (as summarized in Table S2) was pumped into the bottom of the reactor. Ammonium and nitrite, in the forms of $(NH_4)_2SO_4$ and $NaNO_2$, were supplied as substrates. To minimize the interference of nitrite accumulation and free ammonia inhibition, ammonium was supplied in excess with an equimolar amount of nitrite, and the influent pH was maintained at 7.8 ± 0.1. The volumetric nitrogen loading rate (NLR) was controlled by adjusting the hydraulic retention time (HRT) and influent substrate levels based on the operating status of the reactor. The initial HRT and influent total nitrogen (TN) concentration were set at 1.2 h and 560 mgN L^{-1}, respectively. The sludge in the reactor was mainly lost by washout (approximately 20 mgSS L^{-1} at an HRT of 0.96 h), and the sludge that exceeded the working volume was withdrawn from the reactor periodically and for experimental analysis.

Given the environmentally relevant level and the increasing use of ZnONPs, the effects of ZnONPs were tested at three specific sludge loads (0.05, 0.25 and 0.5 mg g^{-1}VSS), based on previous studies (Mu et al., 2012; Mu and Chen, 2011). To be detailed, ZnONPs were added to the influent on Day 31 at a level of 1.0 mg L^{-1} and were then increased to 5.0 mg L^{-1} on Day 46 and 10 mg L^{-1} on Day 61.

2.3. Resistance, resilience and stability metrics

The "amplification envelope" method has been widely used to measure the functional stability of ecosystems (e.g., bioreactors) in response to disturbances (Cabrol et al., 2016; Wells et al., 2017). In this study, the performance deviation of anammox reactors subjected to a perturbation was tracked by using this method. Five shocks of ZnONPs (at 10 mg L^{-1}) were applied to the anammox reactor on Days 61–63, 107, 115, 120–121, and 125–132. The NLR of the reactor was maintained at 14.0 kgN m^{-3} d^{-1} during each shock. The nitrogen removal rate (NRR) of the anammox reactor was defined as the response variable, and the previously unperturbed reactor, which was operated at