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Combined impacts of nanoparticles on anammox granules and the roles of EDTA and S^{2-} in attenuation

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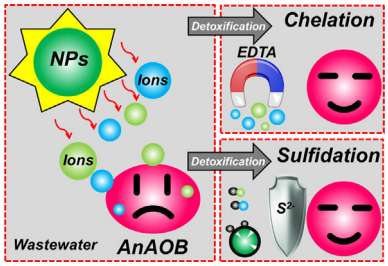
HIGHLIGHTS

- CuNPs at 5 mg g^{-1} SS inhibited anammox activity and damaged the cell membrane.
- CuNPs stimulated N_2H_4 accumulation outside of anammox cells.
- CuONPs or ZnONPs did not significantly affect the toxicity of CuNPs.
- EDTA or S^{2-} attenuates the adverse effects of CuNPs on anammox cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous studies investigating the risk of engineered nanoparticles (NPs) to biological wastewater treatment have primarily tested NPs individually; however, limited data are available on the impact of NPs on the anaerobic ammonium oxidation (anammox) process. In this study, the toxicity of CuNPs on anammox granules was investigated individually and in combination with CuONPs or ZnONPs. Exposure to CuNPs at 5 mg g^{-1} suspended solids (SS) decreased the anammox activity to 47.1 ± 8.5%, increased the lactate dehydrogenase level to 110.5 ± 3.4% and increased the extracellular N_2H_4 concentration by 16-fold but did not cause oxidative stress. The presence of CuONPs or ZnONPs at 5 mg g^{-1} SS did not significantly aggravate or alleviate the toxicity of the CuNPs; however, the introduction of EDTA or S^{2-} could attenuate the adverse effects of the CuNPs, CuONPs and ZnONPs on the anammox granules. EDTA captured Cu ions, whereas S^{2-} shielded and deactivated Cu ions and passivated CuNPs. Therefore, our results indicated that the toxicity of NPs was dependent on the amount of active metal reaching the anammox cells. Overall, the results of this study have filled knowledge gaps and provided insights into the combined toxicity of NPs on anammox biomass.

1. Introduction

With the rapid development of nanotechnology, engineered nanoparticles (NPs) have been widely applied in many fields [1]. For instance, CuNPs are widely used in wood preservation, rubber products, bioactive coatings, microelectronics, textiles and skin products [2]. The distinctive physico-chemical properties of CuONPs and ZnONPs make them valuable in cosmetics, paints, coatings, electronic sensors and solar cells [3]. The extensive applications of NPs have inevitably led to their release into the environment, and recent concerns regarding their potential risk to the environment have been raised. Wastewater treatment plants (WWTPs) are considered one of the important acceptors of these

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released NPs at the end of their lifetime [3,4], and the potential impacts of NPs on the functional bacteria in biological wastewater treatment have been studied extensively. Many studies investigated the impacts of NPs (such as TiO_2, Ag, ZnO) using *Pseudomonas putida* as simplified model of real sludge because these gram-negative and aerobic bacteria are the predominant ones in activated sludge [5–7]. Although these tests using a pure culture instead of a mixed sludge accelerates and amplifies the response of the system to the perturbation of NPs [8], biological wastewater treatment is generally a result of the cooperation of multiple bacteria. Thus, other works directly using mixed cultures as targets may provide better references. For instance, 10 mg L^{-1} CuNPs in wastewater did not show obviously acute toxicity to the ammonia oxidizing bacteria [9], whereas the addition of 17.4 mg L^{-1} CuNPs to anaerobic fermentation reactor inhibited the hydrolysis and acidification process [10], and long-term exposure to 5 mg L^{-1} CuNPs even increased the number of denitrifiers in the sequencing batch reactor [11]. 1.75 mg L^{-1} CuONPs caused acute and chronic inhibition on methanogenesis [12]. And the presence of ZnONPs impacted the process of anaerobic digestion, aerobic nitrification, anoxic denitrification and enhanced biological phosphorus removal to varying extents [13–16]. However, to our knowledge, information is limited regarding the potential risk of NPs to the anaerobic ammonium oxidation (anammox) process [17].

Autotrophic anammox bacteria can directly convert ammonium to dinitrogen gas using nitrite as an electron acceptor in the absence of oxygen and organic carbon. Thus, the anammox process has been widely regarded as a promising alternative to the classical nitrification–denitrification process. Globally, the number of full-scale installations of anammox process had exceeded 100 by early 2015 [18]. Additionally, an increasing number of planning projects are adopting anammox-based processes because the application of anammox for N-removal can turn WWTPs into biofuel factory [19]. However, emerging NPs pose a potential challenge to the anammox process [17].

Although many studies have been performed to determine the potential impacts of NPs on biological wastewater treatment, previous studies have primarily focused on evaluating the effects of individual type of NPs [3,4]. However, because WWTPs are one of the final acceptors of NPs, they are likely exposed to a mixture of NPs, even if the NPs are released at different points [20]. Hence, adequate knowledge on the interactions between different NPs and their combined impacts is of great significance.

Our preliminary study showed that CuNPs made a difference with CuONPs and ZnONPs in the acute toxicity to anammox biomass [17]. Therefore, in this study, these NPs were selected as model NPs to (i) investigate their combined toxicity, (ii) decipher the action mechanism, and (iii) develop possible detoxification strategies. Overall, this study is the first to evaluate the combined toxicity of NPs on anammox granules and represents the first targeted attempt to attenuate their toxicity.

2. Materials and methods

2.1. Origin of the biomass and nanoparticles

The anammox granules used for batch experiments were obtained from a laboratory-scale (2.0 L) up-flow anaerobic sludge blanket (UASB) reactor. This parent reactor, which is fed with synthetic wastewater, has been operating stably under thermostatic (35 ± 1 °C) conditions for more than one year. These anammox granules were dominated by anammox bacteria of the species *Candidatus Kuenenia stuttgartiensis* [21]. The specific anammox activity (SAA), mean diameter and extracellular polymeric substance (EPS) content of the anammox granules used for the batch experiments were 612 ± 44 mg TN g^{-1} volatile suspended solids (VSS) d^{-1}, 2.14 ± 1.1 mm and 262.5 ± 7.9 mg g^{-1} VSS, respectively.

Table 1
Summary of conditions applied to each batch experiment.

Experiment[a]	Reagent addition[b]				
	Cu (mg g^{-1} SS)	CuO (mg g^{-1} SS)	ZnO (mg g^{-1} SS)	EDTA (mM)	S^{2-} (mM)
CK	–	–	–	–	–
Cu	5	–	–	–	–
Cu + CuO	5	5	–	–	–
Cu + ZnO	5	–	5	–	–
EDTA	–	–	–	0.56	–
Cu + EDTA	5	–	–	0.31	
Cu + ZnO + EDTA	5	–	5	0.56	
S^{2-}	–	–	–	–	0.56
Cu + S^{2-}	5	–	–	–	0.31
Cu + ZnO + S^{2-}	5	–	5	–	0.56

[a] All the tests were incubated on an orbital shaker (180 rpm) in the dark at 35 ± 1 °C and the initial pH was fixed at 7.5 ± 0.1. The initial ammonium and nitrite concentrations were both set at 100 mg N L^{-1}.
[b] EDTA and S^{2-} were added in the form of Na_2EDTA and Na_2S, respectively.

Commercially produced CuNPs (10–30 nm), CuONPs (40 nm) and ZnONPs (30 ± 10 nm) of 99.9% purity were purchased from Aladdin Reagent Co. Ltd., China. NP stock suspensions (2 g L^{-1}, pH 7.5) containing 0.1 mM sodium dodecylbenzene sulfonate (SDBS, as a dispersing reagent) were prepared according to a previous study [22]. Previous study reported that the CuNPs (purchased from the same company) were covered by an oxidized layer, which was mainly composed of Cu_2O and a thin outer layer of CuO by X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) analysis [2]. The stock suspensions were sonicated for 1 h in an ultrasonic bath (25 °C, 40 kHz, 250 W) to break aggregates prior to dilution to the exposure concentrations for subsequent experiments.

2.2. Exposure experiment

Batch exposure experiments were performed in a series of reactors (160-mL serum flasks). A total of 100 mL of inorganic synthetic wastewater (Table S1) containing substrates, minerals and trace elements was introduced to the reactors. NP stock suspensions were diluted into each reactor in the concentrations shown in Table 1. The estimated amounts of NPs in wastewater ranged from μg L^{-1} to mg L^{-1}, and their high affinity for sludge may induce the accumulation of NPs [4,23,24]. Our preliminary study tested the acute response of anammox granules to the exposure of CuNPs, CuNPs and ZnONPs in the range of 0.25–50 mg g^{-1} SS, which referred to the loads of previous studies [3,16,25]. The results showed that CuNPs and ZnONPs had no acute toxicity in the testing loads [17], but the acute inhibitory effects of CuNPs on anammox activity could be simulated by the empirical and extended noncompetitive inhibition model (R^2 = 0.9247): $NAA = 100 \times \left(\frac{1}{1+([CuNPs]/4.64)0.82}\right)$, in which the SAA in each experiment was normalized to a control check (CK) that was not exposed to NPs as follows: normalized anammox activity (NAA, %) = $SAA_{NPs}/SAA_{control} \times 100$. Given their wider large-scale production, the potential effects of higher loads of NPs were investigated to reach a final conclusion and seek thorough strategies for attenuation. CuNPs at 5 mg g^{-1} SS, which is close to the estimated IL_{50} (load causing 50% inhibition, 4.64 mg g^{-1} SS), significantly inhibited the activity of anammox granules and thus was chosen for testing in this study.

To reduce the effect of metal ions released from the NPs, two shielding reagents, EDTA and S^{2-}, were added to the wastewater in the form of Na_2EDTA and Na_2S, respectively. Fresh anammox