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Gold and silver nanoparticle effects on ammonia-oxidizing bacteria cultures under ammoxidation

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HIGHLIGHTS

- Nano-silver significantly inhibited bacterial ammoxidation in the aquatic environment.
- The ammoxidation inhibition resulted from AOB biodiversity and abundance reduction.
- The AOB biodiversity and abundance and the ammoxidation were not inhibited by nano-gold.
- Nano-Ag would affect nitrogen cycling but nano-Au would not after entering aquatic environment.

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ABSTRACT

Owing to their wide application in industry and manufacturing, understanding the environmental safety of gold (Au) and silver (Ag) nanoparticles entering aquatic environment is a global issue of concern. For this study, ammonia-oxidizing bacteria (AOB) enrichment cultures reproduced from surface sediments taken from the Jiulong River estuary wetlands (Fujian Province, China) were spiked with nano-Ag and nano-Au to determine their impact on ammoxidation and the mechanisms involved in the process. Results showed that nano-Ag significantly inhibited bacterial ammoxidation in aquatic environment, with the average ammoxidation rate decreasing with increasing nano-Ag concentration. The average ammoxidation rate was significantly correlated to the Shannon index, the Simpson index, and AOB abundance. This suggested that ammoxidation inhibition resulted primarily from AOB biodiversity and abundance reduction, caused by the antibacterial property of nano-Ag. However, AOB biodiversity and abundance as well as bacterial ammoxidation were not inhibited by nano-Au (with a maximum experimental concentration of 2 mg L^{-1}). Moreover, an insignificant correlation was found between AOB biodiversity and abundance and the average ammoxidation rate under the nano-Au treatment. Given that ammoxidation is regarded as a rate-limiting procedure in nitrogen (N) circulation, nano-Ag would affect N cycling but nano-Au would not after entering aquatic environments. Identified nano-Ag and nano-Au impacts on ammonium nitrogen transformation could be generalized in aquatic environment according to their extensive representation in the phylogenetic tree.

1. Introduction

Nanotechnology is advancing rapidly (Roco and Brainbridge, 2001; Gao et al., 2013). Owing to its widespread usage, it is inevitable that nanomaterials will be discharged into the environment during manufacturing and application processes. Subsequently, the risk is they can easily be released into their ambient environment and in turn trigger potential human health and ecological problems (Luo et al., 2011).

Gold and silver nanoparticles (nano-Au and nano-Ag) are metallic ultrafine particles widely used in industrial and manufacturing applications such as coating, textiles, water treatment, catalysts, and biosensors (Zheng et al., 2011a; Li et al., 2012). Previous studies have demonstrated that certain important nanoparticles that include nano-Ag can cause nitrification inhibition via water treatment (Choi and Hu, 2009; Zhang, 2010; Zheng et al., 2011a,b). Additionally, nano-Au can accumulate in the cells of organisms over time and result in toxicity (Huang, 2007; Sereemaspun

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et al., 2008). Nano-Au and nano-Ag can be predicted to also affect N circulation, an important aspect of nutrient cycling. Ammoxidation is considered a rate-limiting procedure in N circulation (Moin et al., 2009). When ammoxidation processes are altered, it is projected that nano-Au and nano-Ag can subsequently affect nutrient absorption of vegetation, eutrophication in aquatic environment and even play a part in greenhouse gas release. Almost all previously published studies on the subject have focused on nanomaterials' influence on nitrification during water treatment processes. Little is known regarding its relative influence on ammoxidation and the mechanisms involved in the process.

Ammonia-oxidizing bacteria (AOB) are one of the primary drivers in ammoxidation processes (Purkhold et al., 2000; Moin et al., 2009). This study focused on identifying how AOB enrichment cultures are influenced by nano-Au and nano-Ag. For this study, changes in ammonium nitrogen ($NH_4^+ - N$) concentrations were measured to determine ammoxidation rates. Additionally, AOB biodiversity and abundance were assessed using molecular fingerprint techniques: PCR–DGGE and qPCR. Correlations between AOB biodiversity and abundance and the ammoxidation rate were ascertained to determine the mechanisms involved in how nano-Au and nano-Ag affect ammoxidation. Results from this study will help contribute to the understanding of how engineered nanomaterials impact other important contaminant behavior in aquatic environment and the subsequent environmental risks involved.

2. Material and methods

2.1. Nano-Ag and nano-Au preparation

All glass ware was soaked in chloroazotic acid for 24 h, distilled twice and blow-dried. 1 mL of 1% (mass) chloroauric acid ($HAuCl_4$, J&K Scientific Ltd.) solution was added to 100 mL ultrapure water and heated to the water boiling point. Acting as both a reducing and capping agent, 2.5 mL of 1% (mass) trisodium citrate ($C_6H_5Na_3O_7$, J&K Scientific Ltd.) solution was then rapidly added while the mixture was vigorously stirred. Nano-Au was subsequently obtained from the mixture under conditions of continued vigorous stirring and boiling until the color of this mixture turned clear. After removing the mixture from the heat source, the nano-Au mixture was stirred continuously for a further 10 min. It was then stored in a brown volumetric flask and kept at 4 °C under darkened conditions until needed for the experiment. Nano-Au concentration was 56.02 mg L^{-1} in the stock solution, measured using inductively Coupled Plasma Mass Spectrometry (ICP-MS, 7500a, Agilent Technologies Inc.). It was well-dispersed and spherical with a diameter varying between 10 and 20 nm (Fig. 1S), identified using a transmission electron microscope (TEM, Hitachi, H-7650).

To reduce Ag nitrate ($AgNO_3$, J&K Scientific Ltd.), sodium borohydride ($NaBH_4$, Sigma–Aldrich Co.) was used as a reductant and polyvinyl alcohol (PVA, J&K Scientific Ltd.) as a dispersant and stabilizer. Initially, 0.3 g of PVA powder was dissolved in boiling ultrapure water and then diluted to a volume of 500 mL. After which, 20 mL of this PVA solution was added to an $AgNO_3$ solution (119 mg L^{-1}). The mixture was stirred to create a homogeneous blend. The nano-Ag stock was then obtained by rapidly adding 200 μL of the $NaBH_4$ solution (14 mM) into the mixture, and stirring for 5 min until the color turned yellow. In order to quantify whether the nano-Ag stock supervened from the unreduced dissolved Ag (Ag^+ ions), Ag^+ ion concentration within the nano-Ag stock was measured using ICP-MS, applying high-speed centrifugation (Wang et al., 2011). In brief, 5 mL nano-Ag stock was centrifuged for 30 min at 10000 rpm. The supernatant was then used to measure dissolved Ag, using ICP-MS. Dissolved Ag was determined to be only approximately 1.5% of the total silver within the stock, indicating that dissolved Ag was virtually reduced to nano-Ag. ICP-MS determined the nano-Ag stock concentration to be 37.08 mg L^{-1}. It was well-dispersed, spherical with the diameter of 10–20 nm (Fig. 1S). The nano-Ag stock was then stored in a brown volumetric flask and kept under darkened conditions at 4 °C.

2.2. AOB enrichment culture

Surface sediments were sampled from the Jiulong River estuary wetlands dominated by mangrove (*Kandelia candel*) (lat 24°26′43.5″N, long 117°54′28.4″E) in Fujian Province, China, in March 2011. Sediments were packed into valve bags and stored in ice boxes before being quickly transferred to the laboratory where they were stored under freezing conditions (−20 °C). AOB enrichment medium composition, provided in Table 1S (Bollmann et al., 2011), was composed of 1.0 g $(NH_4)_2SO_4$, 5.0 g $CaCO_3$, 0.75 g $K_2HPO_4{\cdot}3H_2O$, 0.30 g $MgSO_4{\cdot}7H_2O$, and 0.03 g $FeSO_4{\cdot}7H_2O$. Both the enrichment medium and laboratory wares were sterilized for 30 min at 120 °C. pH value was maintained at 7.5. Each enrichment medium (100 mL) was subsequently added separately into an Erlenmeyer flask (250 mL) under sterile conditions. After which 0.1 g of thawed sediment was separately transferred into Erlenmeyer flasks and shaken for suspension in the enrichment medium. The Erlenmeyer flasks were then placed in a constant temperature incubator at 26 °C under darkened conditions. To resuspend sediment, each Erlenmeyer flask was shaken for one min every two d throughout incubation. Initial AOB enrichment cultures were obtained after reproducing for 140 d and undergoing six transfers. Then cultures were inputted into the fresh enrichment medium with the volume ratio of 1/9 (cultures to the fresh enrichment medium). Finally, AOB enrichment cultures needed in this experiment were obtained after secondary propagation. In these enrichment cultures, ammonia-oxidizing archaea (AOA) were not identified in keeping with previously reported methodology (Yu et al., 2011) that agree that ammoxidation in the test solution was dominated by AOB activity.

2.3. Nanomaterial treatments and sample analysis

Potential concentrations in environmental matrices are believed to be those that vary from 0 μg L^{-1} to 2 mg L^{-1} (Gottschalk et al., 2009). In this study, nanomaterial (nano-Ag and nano-Au) stock solution concentrations to which cultures were exposed were as low as 50 μg L^{-1} (finial concentration) and as high as 2 mg L^{-1} (finial concentration). Each treatment contained 5 mL bacterial suspension to which 30 mL fresh nutrient-rich medium and 5 mL nanomaterial solution of different concentrations were added. Subsequently, the finial ammonium ($NH_4^+ - N$) concentration in the test solution was approximately 204 mg L^{-1}. Additionally, a 1 mL saturated solution of potassium chlorate ($KClO_3$) was then added to the mixtures to inhibit (NH_4^+) to nitrite oxidation. Concurrently, a control group of 5 mL ultrapure water was established with no nanomaterial solution added to the mixtures. Each treatment was carried out in triplicate in 150 mL Erlenmeyer flasks. All treatment flasks were placed in a constant temperature incubator under darkened conditions at 28 °C for 45 d. During the incubation period, cultures were sampled five times on day 3, 10, 17, 31, and 45. Samples were immediately strained through a filter membrane (0.22 μm), and filtrate was collected to measure ($NH_4^+ - N$) concentration using a flow injection analyzer (Lachat QC8500) and the manufacturer's method with the QuikChem Method 10-107-06-1-B (Smith, 2001). Meanwhile, filter residue was collected to determine diversity to *amoA* using PCR-DGGE and abundance to 16S rRNA using AOB qPCR (Yu et al., 2011). Primer pairs amoA-1F (GGGGTTTCTACTGGTGGT) and amoA-2R-TC

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