Introduction

Silver nanoparticle (Ag NP) usage as a biocide in consumer goods (clothing, etc.) has increased drastically since 2006 (1). Research has indicated that some leaching may occur when these articles are used or cleaned (2, 3). This leads to Ag NP in wastewater treatment plant (WWTP) influent and WWTP processes. Ammonia oxidizing bacteria (AOB) perform a very important step of WWTP processes, the oxidation of ammonia (NH$_3$) to nitrite (NO$_2$). This presented research studied Ag-NP-induced inhibition of Nitrosomonas europaea, a model AOB, in a variety of aquatic chemistries. Five types of silver were used as inhibitors: Ionic silver (Ag$^+$); 20 nm citrate-stabilized BioPure Ag NP; 20 nm and 80 nm phosphate-stabilized Ag NP and PVP-stabilized 3-5 nm Ag NP (nanoComposix Inc., San Diego, CA). Protection from Ag-NP stability and aggregation were measured in abiotic experiments.

Materials and Methods

Inhibition of N. europaea by Ag$^+$ and Ag NP

To create stable solutions of dilute Ag NP in media with high ionic strength, Ag NP were dispersed in distilled deionized water (DDI H$_2$O) in test bottles shaken at 250 rpm. After 30 min, a concentrated test media solution was added, to final concentrations of 30 mM HEPES (pH 7.8) and 2.5 mM (NH$_4$)$_2$SO$_4$ and shaken again for 30 min, a concentrated test media solution was added, to final concentrations of 30 mM HEPES (pH 7.8) and 2.5 mM (NH$_4$)$_2$SO$_4$ and shaken again for 30 min. N. europaea cells were added to a concentration of ~7 mg/L and shaken at 250 rpm, the solutions were filtered using 10 μm syringe filter (for the 80 nm Ag NP) and Ag$^+$ concentrations were measured every 45 min using a colormetric assay.

Ag NP Dissolution in Test Media

Leaching of Ag$^+$ from Ag-NP in test media (30 mM HEPES (pH 7.8), and 2.5 mM (NH$_4$)$_2$SO$_4$) was measured at varying concentrations of Ag NP. After shaking diluted concentrations of Ag NP for 3 h at 250 rpm, the solutions were filtered using either ultracentrifugation (5kDa pore volume) or a 20 nm syringe filter (for the 80 nm Ag NP). Ag$^+$ concentrations were measured both in the unfiltered solution and the filtrate using the ICP-EOS.

K$^+$ release in the Presence of Ag$^+$ and Ag NP

K$^+$ release was measured by shaking control and treatment bottles for 3 h at 250 rpm and then followed by a pelleting of the cells. K$^+$ in the supernatant and in the pellet cells dissolves for 18 h in 3 N HNO$_3$ was measured using the ICP-EOS.

Stability and Aggregation of Ag NP in Test Media

Ag-NP stability and aggregation were measured with dynamic light scattering (DLS) in the presence of various test media. Average hydrodynamic radius of Ag-NP in suspension was measured for 1 h.

Results

Nanoparticle Dissolution

Figure 1

In the presence of 2.5 mM (NH$_4$)$_2$SO$_4$ and 30 mM HEPES (pH 7.8), 9.9 μM FeSO$_4$, and 0.65 μM CuSO$_4$ (A), 730 μM MgSO$_4$ (B), 730 μM CaCl$_2$ (C), and 200 μM Ca$_2$(SO$_4$)$_2$ (D).

Conclusions

1. Smaller Ag NP are more inhibitory than larger Ag NP and may be just as inhibitory as Ag$^+$ (Figure 1A). This inhibition largely comes from Ag$^+$ that have leached from the Ag NPs (Figures 1B & 1C).

2. N. europaea cell membranes can be destabilized by exposure to Ag$^+$ and Ag NP, as shown by intracellular K$^+$ release (Figure 2A & 2B).

3. N. europaea can be protected from Ag$^+$ and Ag NP-induced inhibition to an extent by various aquatic bioconstituents, most reliably by Mg$^{2+}$ and Ca$^{2+}$ (Figure 3).

References


Acknowledgements

I would like to thank Dr. Tyler Radniecki and Dr. Lewis Semprini for their membership during this project and Margaret Schneider and Anees Sardari for their assistance in performing experiments. I would also like to thank Dylan Stankus for the data concerning Ag NP aggregation. Funding provided by the Subsurface Biosphere Initiative.