The Role of Trace Metals in Protecting *Nitrosomonas europaea* From Silver Ions and Silver Nanoparticles

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Introduction

Nitrosomonas europaea is an Ammonia-Oxidizing Bacteria (AOB) and contributes to wastewater treatment by removing nitrogen from wastewater.

The process of oxidizing ammonia is expressed by the following equation:

$$\textbf{AOB:}\quad NH_3 + O_2 \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 4e^- + 5H^+$$

Unfortunately, *N. europaea* is often inhibited by compounds found in the wastewater such as silver ions (Ag^+).

Silver nanoparticles (Ag NPs) are particles of silver that are less than 100 nm in diameter (**Figure 1**). Due to their small size, Ag NPs possess many unique properties that have made them an ideal broad spectrum biocide.

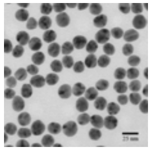


Figure 1. TEM image of Biopure Silver Nanoparticles, courtesy of Nano Composix , Inc.

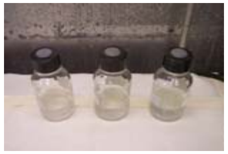
The use of Ag NPs has grown exponentially over the past couple of years as they have found their way into clothing, plush baby toys, food containers, toothpaste, dishwashers and clothes washers.

The widespread use of Ag NPs will potentially lead to significant quantities of Ag^+ and/or Ag NPs being deposited in the wastewater stream and ultimately in wastewater treatment plants. This may inhibit nitrifying bacteria, such as *N. europaea*, which may lead to excess nitrogen being released into the environment causing eutrophication of the receiving body of water.

The goal of this study is to analyze how trace metals found in AOB growth media influence the inhibition of *N. europaea* exposed to Ag^+ and Ag NPs.

Methods

Cells were grown to the late exponential growth stage (3 days). They were harvested, washed and inoculated into triplicate batch bottles containing 15mM NH_4^+, 20mM HEPES buffer (pH 7.8), and various concentrations of Ag^+, Ag NPs, and growth media metals ($MgSO_4$, $CaCl_2$, $FeSO_4$ and $CuSO_4$).



Triplicate batch bottles

The bottles were shaken for 3 hours at 250RPM, in the dark at 30°C. Samples were taken every 45 minutes in duplicate. Nitrite levels were measured using a colorimetric assay. All Ag^+ and Ag NP levels were measured using an ICP-ES.

Ag^+ Results

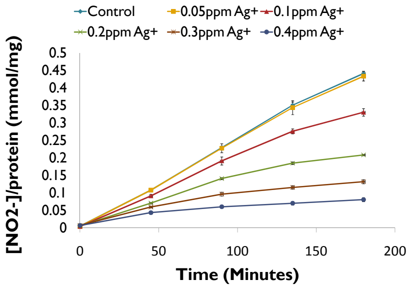


Figure 2. Concentration of nitrite over time for varying concentrations of Ag^+ (with trace metals present).

Production of nitrite decreases as the concentration of Ag^+ increases.

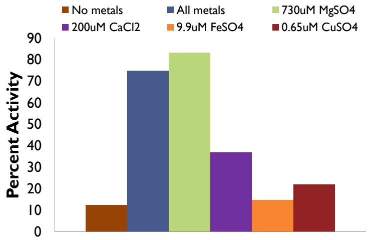


Figure 3. Percent activity of *N. europaea* exposed to 0.1ppm Ag^+ and various trace metals.

Percent activity increased for individual Mg^{2+}, Ca^{2+}, Fe^{2+}, and Cu^{2+} additions as well as the combination of all metals in the media.

Ag NP Results

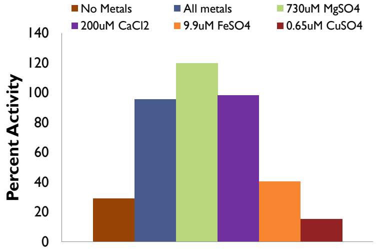


Figure 4. Percent activity of *N. europaea* exposed to 1.6ppm 20nm Ag NPs and various trace metals.

Percent activity increased for individual Mg^{2+}, Ca^{2+}, Fe^{2+}, and Cu^{2+} additions as well as the combination of all metals in the media.

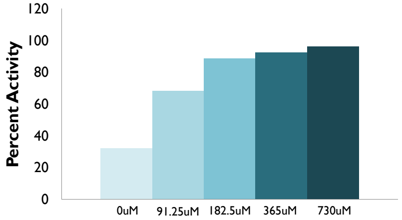


Figure 5. Percent activity of *N. europaea* exposed to 1.6ppm 20nm Ag NPs and varying amounts of Mg^{2+}.

Percent activity increased as the concentration of Mg^{2+} increased.

Ag NP Aggregation

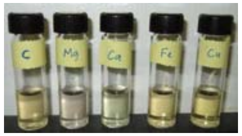


Figure 6. A color change was observed in growth media containing 20nm Ag NPs and either 730 µM $MgSO_4$ or 200 µM $CaCl_2$.

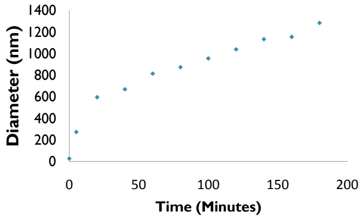


Figure 7. Average diameter of 20nm Ag NPs in 730 µM $MgSO_4$ over time as measured by dynamic light scattering (DLS).

Over time, the 730 µM $MgSO_4$ caused the Ag NPs to grow in diameter.

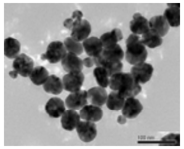


Figure 8. TEM image of 80nm Ag NPs after 3 hrs of exposure to 730 µM $MgSO_4$.

Conclusions

1) *N. europaea* is more sensitive to Ag^+ than Ag NPs.

2) Both Mg^{2+} and Ca^{2+} protected *N. europaea* most effectively from Ag^+ and Ag NPs.

3) Mg^{2+} and Ca^{2+} protected *N. europaea* from Ag^+ potentially by blocking the entrance into the cells, thereby decreasing the negative effects of Ag^+.

4) Mg^{2+} protected *N. europaea* against Ag NPs by making the particles bigger thereby increasing the surface area to volume ratio of the particles making them less able to interact with the cells and release Ag^+. Mg^{2+} may have also blocked the Ag^+ released by the Ag NPs.

Acknowledgements

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