The effect of BSA and Na-Alginate on silver ion inhibition

Danyelle Webb, Tyler Radniecki, and Lew Semprini
School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, OR 97331

Introduction

Ammonia Oxidizing Bacteria (AOB) are considered to be one of the most environmentally sensitive bacteria in waste water treatment plants (WWTP) and surface waters. Because of this, they are ideal for studying the effects of chemical toxicity. The AOB *Nitrosomonas europaea* grows in marine, brackish, and freshwater ecosystems. *N. Europaea* is a large part of the nitrogen cycle. It contributes to the nitrogen cycle by converting ammonia (NH_3) to nitrite (NO_2^-) .

The process of oxidizing ammonia by *N. europaea* is expressed by the following equation:

AOB: $NH_3 + O_2 \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 4e^- + 5H^+$ [1]

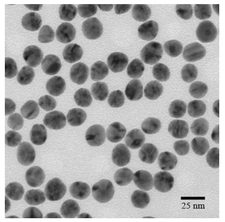


Figure 1. 20nm citrate stabilized Silver Nanoparticles.

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. Silver nanoparticles are the most widely used engineered nanomaterial in costumer products [3].

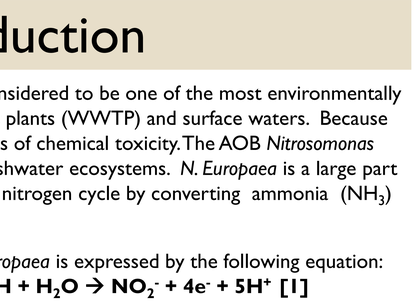
Ag-NPs have found their way into clothing, baby toys, food containers, toothpaste, dishwashers, clothes washers, and other applications [2]. Their widespread use causes them to be released into the environment, eventually ending up in WWTP. The use of Ag-NPs can potentially lead to significant quantities of Ag+ and/or Ag-NPs being deposited in wastewater streams and waste water treatment plants (between 4ppb and 1ppm) [3].

There is a wide variety of organic matter found in WWTP, such as proteins and polysaccharides. Bovine Serum Albumin (BSA) is employed as a representative protein. Sodium Alginate (Na-Alg) was employed as a representative polysaccharide.

The goal of this study is to determine whether BSA and Sodium Alginate protects *N. europaea* from silver nanoparticles and ions

Method 1: Silver Ions

Cells were grown to the late exponential growth stage (3 days). Then they were harvested, washed and inoculated into triplicate 155mL batch bottles. Each bottle contains a media comprised of 30mM Hepes and 2.5mM $(NH)_2SO_4$, and has a working volume of 35mL. The target concentration of cells is ~7mg/L. After adding various concentrations of BSA/Na-Alginate and silver ions the bottles are shaken at 250rpm in a 25°C dark room for 3 hours.



Triplicate batch bottles

Colorimetric Nitrate Assay

A colorimetric nitrite assay is used to determine nitrification activity. Samples were taken every 45 minutes. 10µL of sample is placed in a small microcentrifuge tube containing 890µL sulphanilamide and 100µL NED. The colorimetric nitrate assay is then analyzed with a spectrometer at 540nm. At the end of the experiment each bottle's contents is analyzed at 600nm to normalize cell concentration.

BSA Results

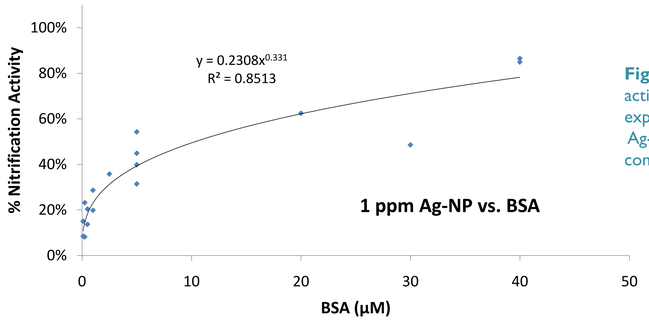


Figure 2. Percent activity of *N. europaea* exposed to 1ppm Ag-NP and various concentrations of BSA

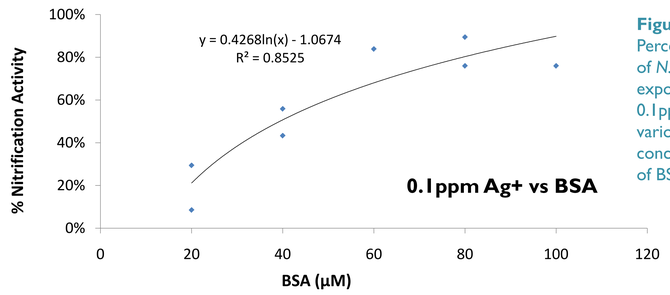
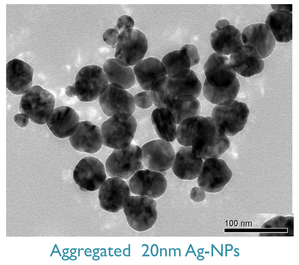


Figure 3. Percent activity of *N. europaea* exposed to 0.1ppm Ag^+ and various concentrations of BSA

Method 2: Silver Nanoparticles



Aggregated 20nm Ag-NPs

Silver Nanoparticles stick to surfaces very easily, including each other, because they have a high ionic strength. When using Ag-NPs a different method must be used because the silver nanoparticles have a tendency to aggregate together unless introduced to the media in a way that prevents aggregation.

First, each bottle is filled with 32.67mL of deionized water and the silver nanoparticles are added. Then the bottles are placed on the shaker for 15 minutes. This step is critical to prevent aggregation of the Ag-NPs, leading to inconsistent results.
After shaking for 15 minutes 2.33mL of 15x concentrated media is added to each bottle for a final concentration of 30mM Hepes and 2.5mM $(NH)_2SO_4$) as well as the BSA or Alginate. The bottles are then placed back on the shaker table for 20 minutes at 250rpm at 25 °C. After 20 minutes cells are added to a concentration of ~7mg/L. A colorimetric Nitrate Assay is used to analyze samples.

Sodium Alginate Results

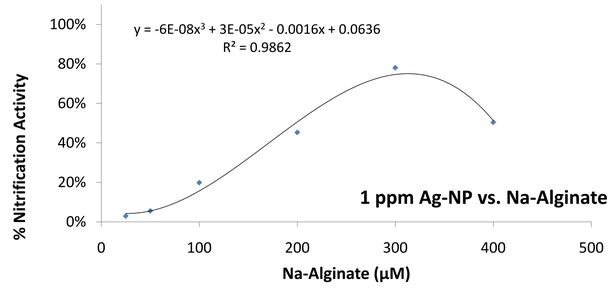


Figure 4. Percent activity of *N. europaea* exposed to 1ppm Ag NP and various concentrations of Na-Alginate

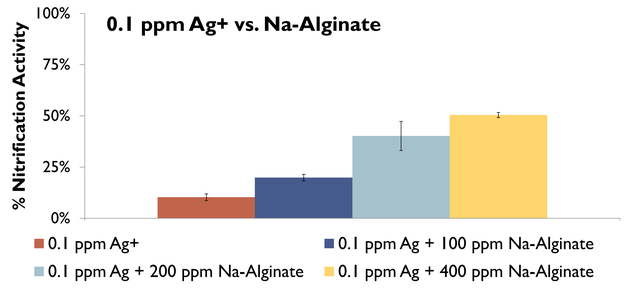


Figure 5. Percent activity of *N. europaea* exposed to 1ppm Ag-NP and various concentrations of Na-Alginate

Conclusions

1) *N. europaea* is protected by BSA, presumably because the BSA proteins bond with the silver ions.

2) N. europaea is protected by high concentrations of sodium alginate. One theory is that, because sodium alginate is composed of heteropolysaccharides it can absorb Ag-NP and potentially inhibit their dissolution to form Ag^+.

3)Cells were much less inhibited by Ag-NP compared to Ag^+ indicating the silver ion is mainly causing the inhibition and toxicity.

References

[1] Radniecki, T., and Lauchnor, E., Investigating Nitrosomonas Europaea Stress Biomarkers in Batch, Continuous Culture, and Biofilm Reactors. *Methods in Enzymology.* Elsevier Academic. (2011) pp 217-46.

[2] Liu, J., and Hurt, R. Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids. *Environmental Science & Technology* 44.6 (2010): 2169-175.

[3] Radniecki, T. et al. Influence of liberated silver from silver nanoparticles on nitrification inhibition on *Nitrosomonas europaea*. *Chemosphere* (2011) doi:10.1016/J.2011.06.039.

Acknowledgements

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