Investigating the Toxicity of Silver Ions to Chronically Exposed Nitrifying Bacteria

Issa El Haddad, Kevin Csupak, Tyler S Radniecki

Department of Civil, Construction and Environmental Engineering, San Diego State University, San Diego CA 92182

Introduction

As silver has been known to inhibit the growth of bacteria and fungi, it became a widely used component in consumer-based products in the form of silver nanoparticles (Ag-NPs), particles of less than 100 nm in diameter (Figure 1). Ag-NPs exhibit a high surface area to volume ratio, and thus are highly reactive and dissolve into silver ions (Ag^+) over a short period of time.

Due to the increased use of Ag-NPs in consumer products, the implications of increased concentrations of silver reaching wastewater treatment plants (WWTPs) is a concern to environmental engineers. At the WWTPs, the model ammonia oxidizing bacteria, *Nitrosomonas europaea* (Figure 2), plays a key role in the removal of nitrogen from the wastewater, in a process called nitrification. Nitrification is the biological oxidation of ammonia (NH_3) to nitrite (NO_2^-).

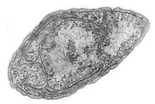


Figure 2. *Nitrosomonas europaea*

Previous studies have shown that *N. europaea* are very sensitive to acute exposures (3h) to both Ag^+ and Ag-NPs at relatively high concentrations. This study explores the sensitivity of *N. europaea* chronically exposed to low concentrations of Ag^+.

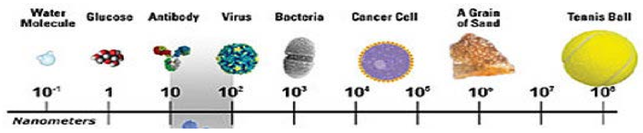


Figure 1. Nanoparticles scale.

Objective

The central hypothesis of this study is that low concentrations of Ag^+ will become lethal to chronically exposed *N. europaea*, due to an accumulation of Ag^+ in the cell fraction of the reactors.

Methods

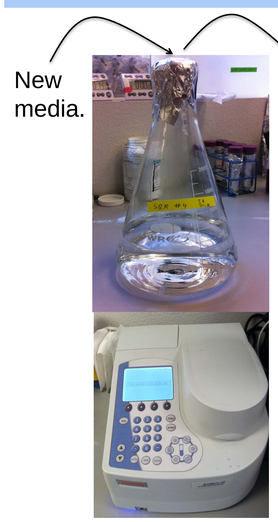


Figure 3. Sequencing Batch Reactors (SBRs).

SBRs setup:

- 6 1L Erlenmeyer flasks, 3 control and 3 variables.
- Cultured with *N. europaea.*
- Shaken at 110 rpm at 30°C in the dark.

Daily Procedure:

- Volume is kept constant by adding and removing 120 mL of media (Figure 3).
- Ag^+ doses added.
- pH adjusted using 10N NaOH.
- Samples measured using UV-Vis (Figure 4).

Figure 4. UV-Vis measures OD_{600} and NO_2^- production.

Results

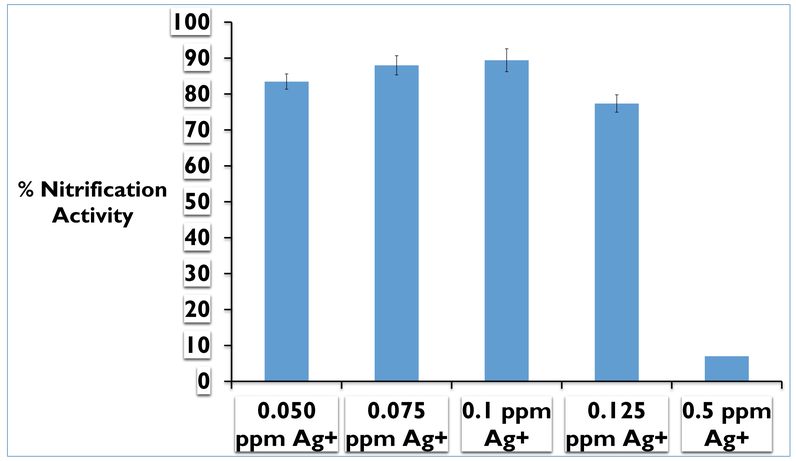


Figure 5. Percent nitrification activity of continuously cultured *N. europaea* cells exposed to Ag^+.

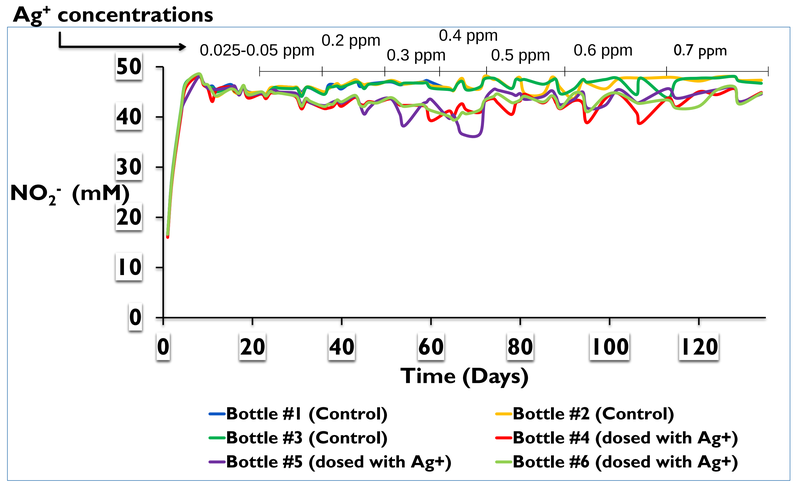


Figure 6. Nitrite produced over the course of the experiment.

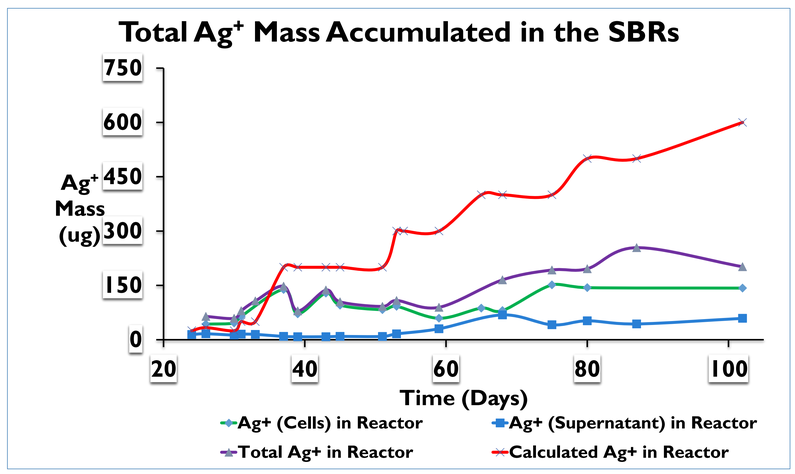


Figure 7. Total Ag^+ mass accumulated in the SBRs after running a mass balance using the ICP-MS.

Conclusions

1) *N. europaea* cells in 3h batch assays were completely inhibited by 0.5 ppm Ag^+ (Figure 5), but were not inhibited by 0.7 ppm Ag^+ in the SBRs (Figure 6). Future experiments are being conducted to determine the cause.

2) *N. europaea* cells showed only a slight decrease in NO_2^- production, even at concentrations as high as 0.7 ppm (Figure 6). It is unknown why the cells show such high tolerance to Ag^+ in the SBRs, but possible factors include the presence of trace metals and the slower growth rates of cells in the SBRs compared to the simplified test media and exponentially growing cells used in previous acute batch assays.

3) The mass of Ag^+ found associated with *N. europaea* cells increased throughout the experiment as the concentration of Ag^+ added to the SBR media increased (Figure 7). However, and as indicated in Figure 6, this amount of adsorbed Ag^+ was not enough to severely inhibit *N. europaea*.

4) The concentration of Ag^+ in the SBR supernatant (i.e. the Ag^+ not associated with the cell mass) did not increase with the increasing Ag^+ dosing, which led to a poor Ag^+ mass balance (indicated by the gap between Total Ag^+ and the Calculated Ag^+ in Figure 7).

Future Research

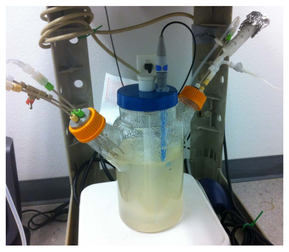
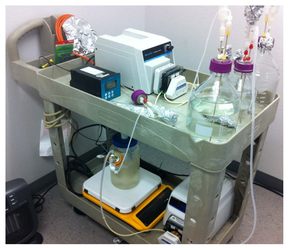


Figure 8. Chemostat with cultured *N. europaea.*

- Investigate the toxicity of Ag^+ and Ag-NPs to chronically exposed *N. europaea* cells in a continuous growth chemostat reactor (Figures 8-9).



- Optimize a more efficient mass balance mechanism to quantify the Ag mass accumulated on the cells in the reactor.

Figure 9. Chemostat setup.

Acknowledgements

This project would not have been possible without funding from the National Science Foundation Grant no.NSF CBED-EHSN #1067572. The project was also supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-38422-31204 from the USDA National Institute of Food and Agriculture. Many thanks and appreciation to Dr. Tyler Radniecki for his guidance and support on this project, and to my colleagues Cameron Kostigen Mumper and Anna-Lucia Uribe for their help and advice in running experiments.