Inquiries into Nitrification Inhibition in *Nitrosomonas europaea*

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Cadmium and Zinc based Inhibition in *N. europaea*

Introduction:

The effects of heavy metals on living organisms are well documented by numerous studies that attest to their significant toxicity. *N. europaea* oxidizes ammonia, a common wastewater constituent, to nitrate which is subsequently reduced to harmless nitrogen gas. However, nitrifiers are particularly sensitive to inhibition due to heavy metals, such as cadmium and zinc. SOUR (specific oxygen uptake rate) tests were employed to determine differences in inhibition based on varying concentrations of the substrate, ammonia, and heavy metal used.

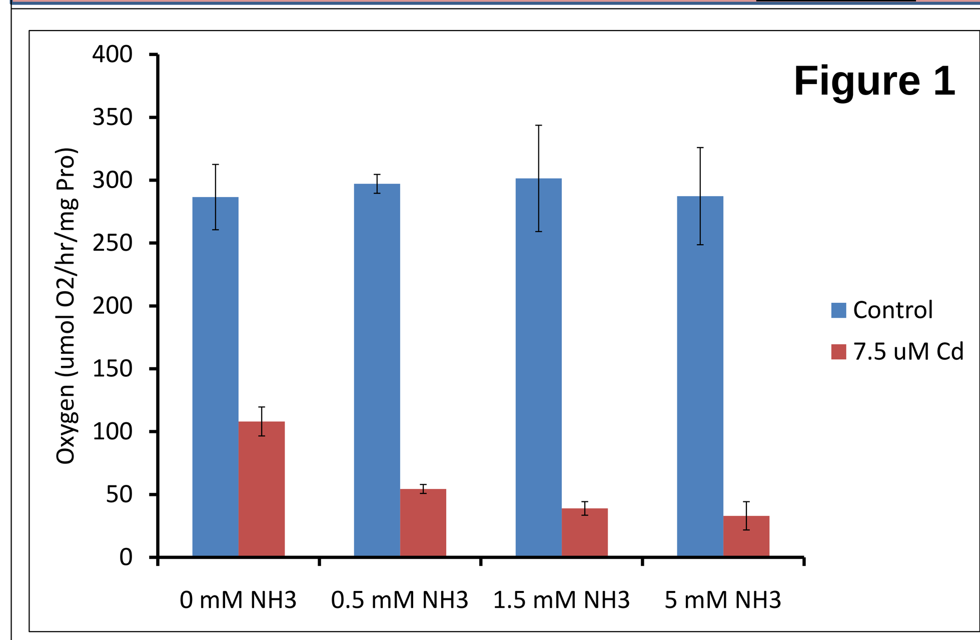
Goals:

- To determine the inhibitory effects of the heavy metals cadmium and zinc on the nitrification activity of *N. europaea* due to different concentrations of ammonia.
- To determine the physiological effect of those same heavy metals on *N. europaea* by tracking the oxygen consumption rates when under inhibition.

Method:

The experiment was performed in 155 mL Wheaton bottles fitted with butyl rubber septa. Each bottle contained 50 mL of media, as well as constant concentrations of cadmium and zinc. Bottles were inoculated with 7 mg protein/L of *N. europaea*. Triplicate bottles were created for each experimental ammonia condition and bottles with no ammonia were used as controls. Samples were taken from all bottles at various time points and were tested for nitrite accumulation as well as the rate at which oxygen was consumed via the SOUR apparatus.

Results



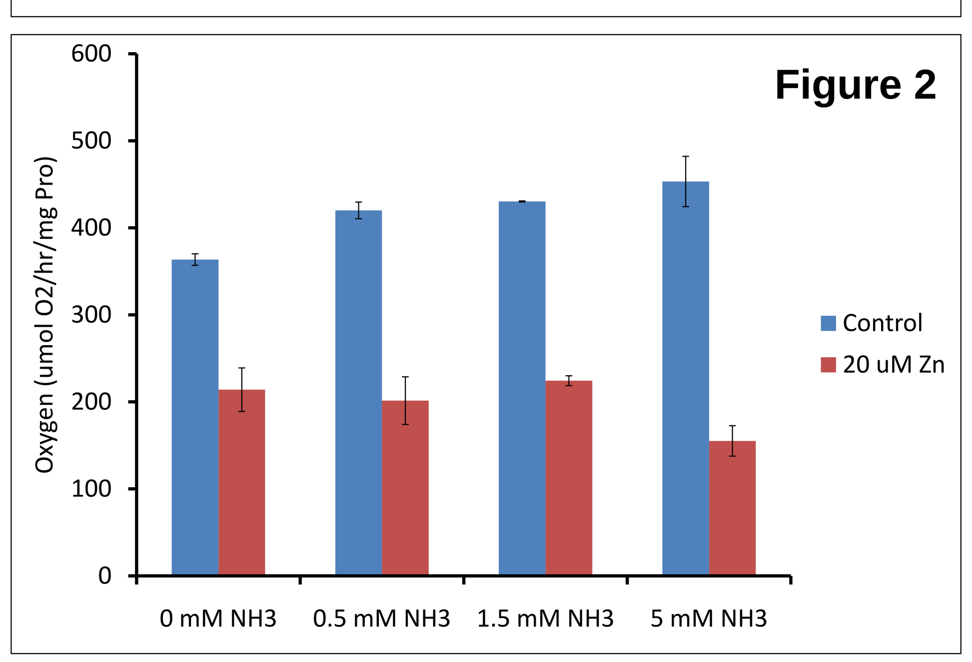


Figure 1: Oxygen consumption at different concentrations of ammonia for cadmium.
Figure 2: Oxygen consumption at different concentrations of ammonia for zinc.

- The SOUR test measured the rate of oxygen consumption by the bacteria.
- Cadmium caused lesser nitrification activity when ammonia was present in contrast to when it was not. Ammonia concentration did not have a significant effect on nitrification inhibition.
- When ammonia was present at any tested concentration of zinc, the reduction of nitrification activity showed no statistically significant differences.

Conclusions:

1. Although the tests for both cadmium and zinc had a similar inhibitory effect after 60 minutes of exposure, there were differences in the post-wash recovery profiles.
2. Cells affected by cadmium showed greater overall inhibition during the post-wash recovery than those affected by zinc, however, there was a distinct decrease in inhibition due to cadmium when ammonia wasn't present.
3. Zinc showed approximately 50% oxygen uptake compared to controls, however there was no significant difference between any of the ammonia concentrations tested.

Corexit 9500A and *N. europaea*

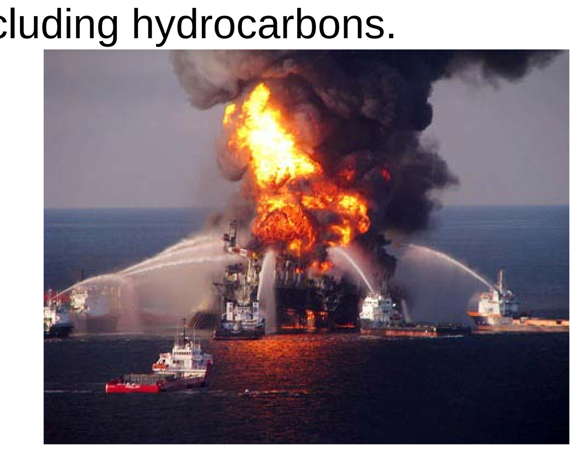
Introduction:

Several months ago, the Deepwater Horizon oil rig in the Gulf of Mexico became the subject of a massive malfunction, causing millions of gallons of crude oil to spill into the surrounding water. A dispersant, known as Corexit 9500A has been applied to the oil in order to break it up and potentially make it easier to degrade and to prevent the oil from reaching the coastline.

Ammonia oxidizing bacteria, similar to *N. europaea,* are an integral part of many marine ecosystems and are an important part of the global nitrogen cycle, but at the same time are very sensitive to low quantities of various toxins, including hydrocarbons.

Goals:

- To find the extent to which Corexit 9500A affects *N. europaea.*
- To find the extent to which crude oil (ANSC) affects *N. europaea.*
- To find out how the comibnation of ANSC and Corexit 9500A affects *N. europaea.*



Method:

The experiment was performed in 155 mL Wheaton bottles fitted with butyl rubber septa. Each bottle contained 50 mL of media, was inoculated with ~7 mg protein/L of *N. europea,* as well as various ratios of oil to dispersant. Triplicate bottles were created for each experimental condition. Samples were taken from the bottles at various time points and spectrophotometry was employed to determine levels of nitrification activity.

Results:

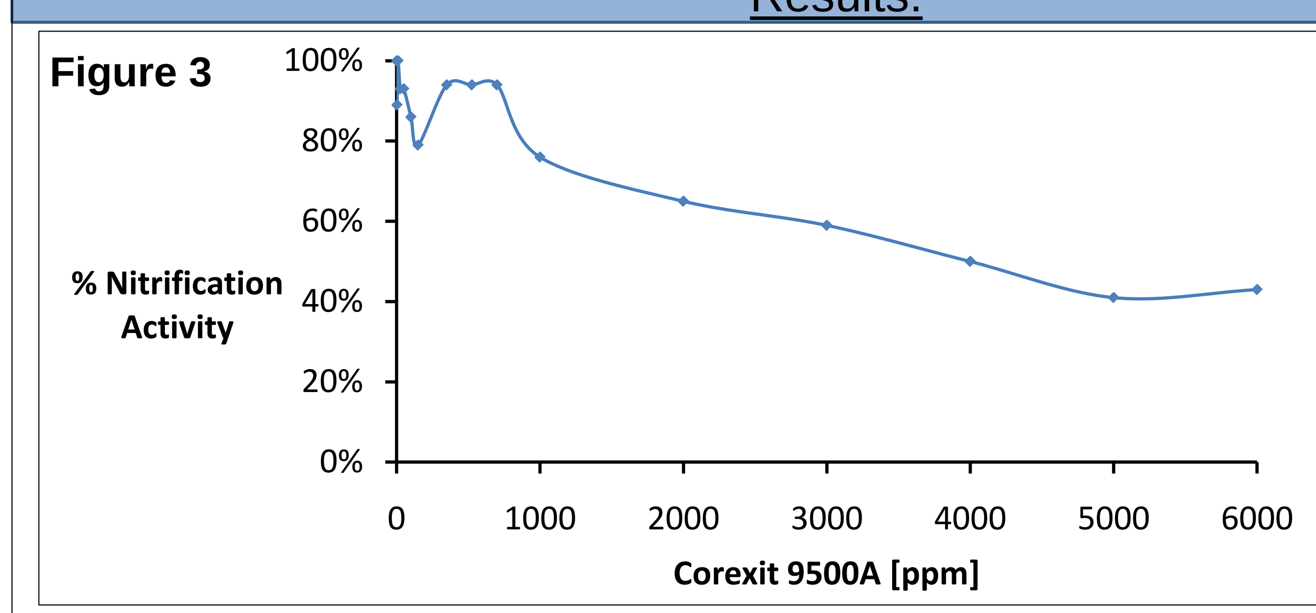


Figure 3: Nitrification activity measured against dispersant concentration

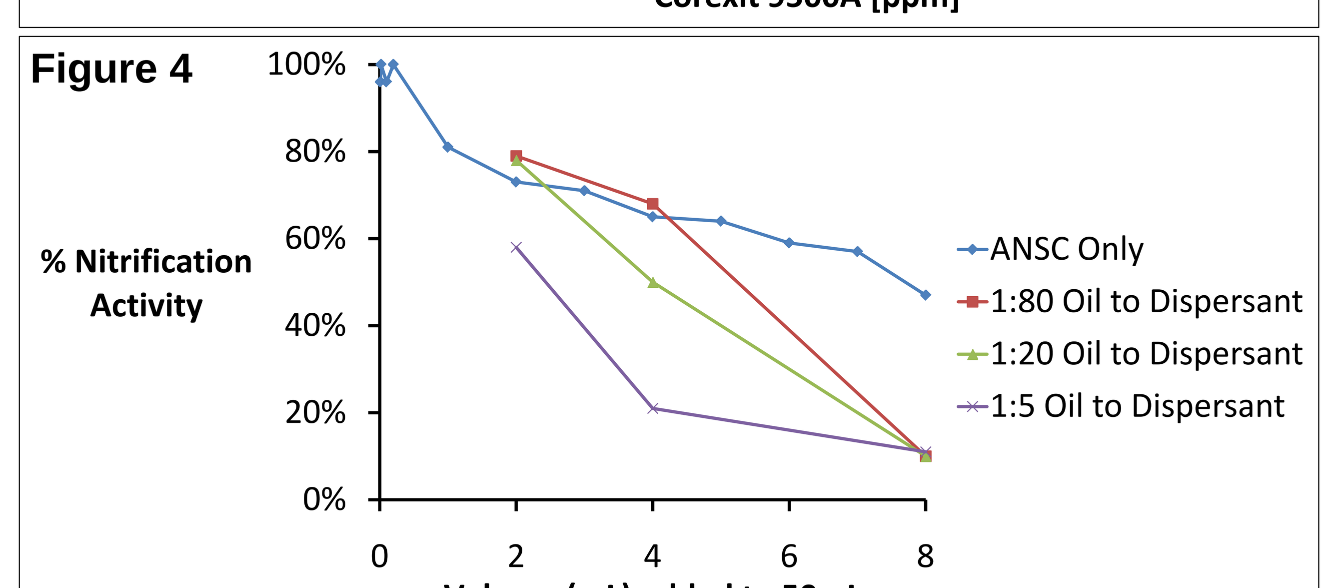


Figure 4: Nitrification activity measured against various ratios of oil to dispersant

Conclusions:

1. Exposing *N. europaea* to the dispersant Corexit 9500A significantly decreases nitrification activity, with the greatest amount occurring at 5000 ppm.
2. The combination of ANSC and Corexit 9500A proved to be even more toxic and inhibitory towards the nitrification activity of *N. europaea*, increasing ANSC's toxicity 5-fold.

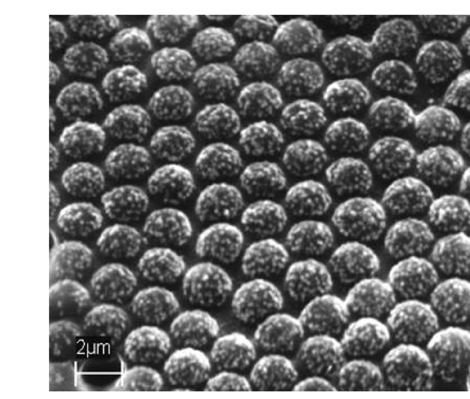
Ionic Silver and Inhibition in *N. europaea*

Introduction:

Nanoparticles have a wide range of uses and are being found in more and more everyday products. However, these same nanoparticles are being increasingly found in the influent streams in wastewater treatment plants which bears asking how they are affecting the ammonia oxidizing bacteria that are crucial in removing ammonia from wastewater. It has been found that the release of ionic silver is responsible for the inhibition induced by silver nanoparticles.

Goals:

- To find the extent to which exposure to silver ions inhibits the nitrification capability of *N. europaea*.
- To test $MgSO_4$, $FeSO_4$ and EDTA for their role in protecting *N. europaea* from silver ion induced inhibition.



Method:

The experiment was performed in 155 mL Wheaton bottles fitted with butyl rubber septa. Each bottle contained 50 mL of media and were all inoculated with ~7 mg protein/L of *N. europaea.* Triplicate bottles were created for each experimental condition. A 0.1 ppm concentration of silver ions was added to treatments of triplicate bottles along with pre-determined concentrations of $MgSO_4$, $FeSO_4$ and EDTA. Samples were takens every 45 min to measure nitrite production.

Results:

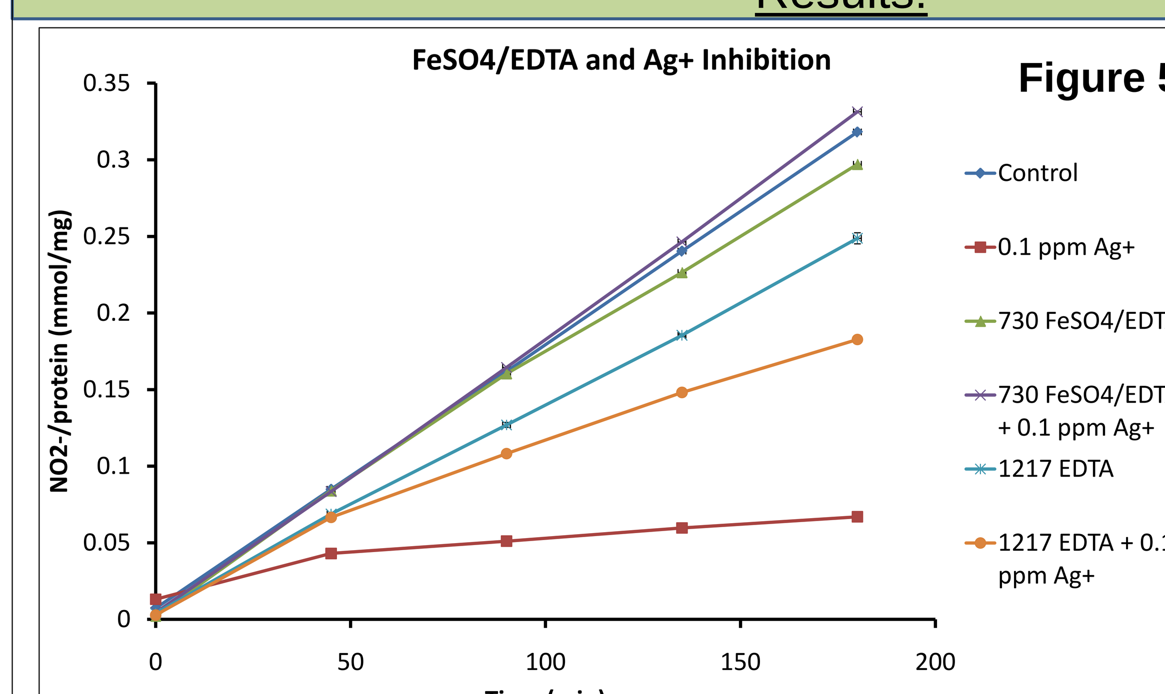


Figure 5: Nitrite accumulation over time for various concentrations of $FeSO_4$ and EDTA after exposure to silver ions.

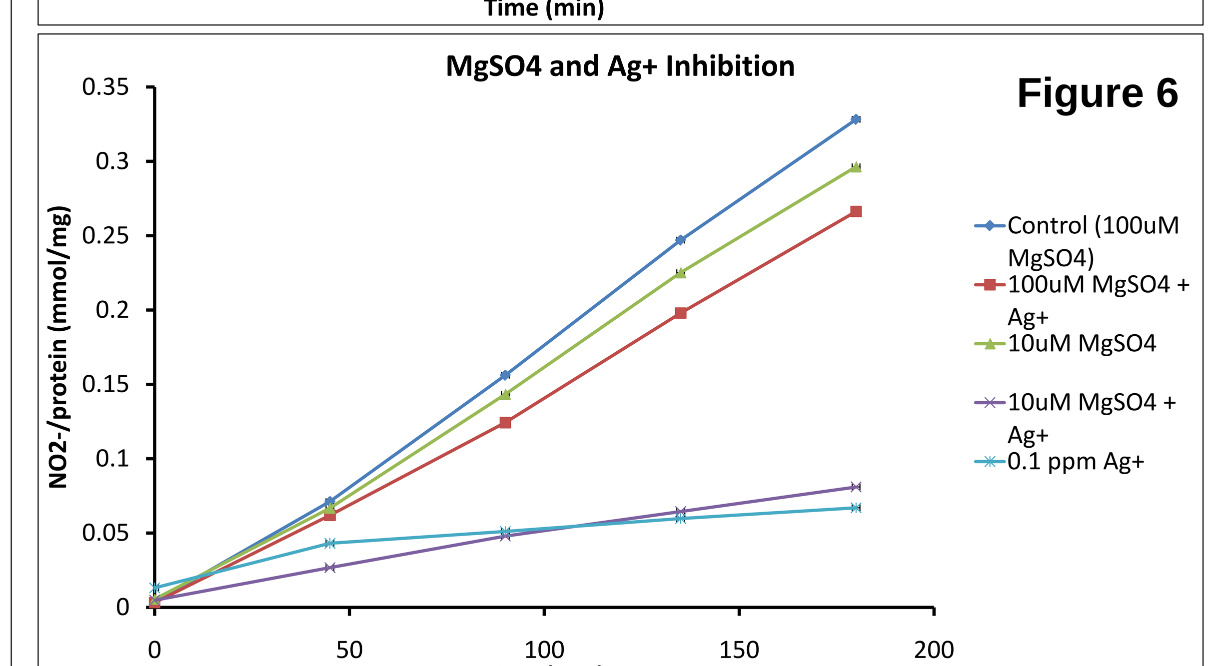


Figure 6: Nitrite accumulation over time for various concentrations of $MgSO_4$ after exposure to silver ions.

Conclusions:

1. Silver ions by themselves severely inhibit the nitrification capability of *N. europaea.* Nitrite accumulation is reduced significantly over long periods of time.
2. Certain concentrations of $FeSO_4$/EDTA and $MgSO_4$ protect *N. europaea* from the silver ions, keeping nitrification at control levels. Specifically, 730 μM $FeSO_4$/EDTA and 100 μM $MgSO_4$ showed the highest levels of nitrite accumulation.

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