Introduction

Ammonia Oxidizing Bacteria (AOB) are considered to be one of the most environmentally sensitive bacteria in wastewater 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 (NH₃) to nitrite (NO₂⁻). The process of oxidizing ammonia by N. europaea is expressed by the following equation:

\[ \text{NH}_3 + \text{O}_2 \rightarrow \text{NH}_2\text{O} + \text{H}_2\text{O} \]

Triplet batch bottles and various concentrations of Na-Alginate

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 consumer 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].

Method 1: Silver Ions

Cells were grown to the late exponential growth stage (3 days). Then they were harvested, washed and inoculated into triplicate 155 mL batch bottles. Each bottle contains a media comprised of 30mM Hepes and 2.5mM (NH₄)₂SO₄ as well as the BSA or Alginate. 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 (NH₄)₂SO₄ 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.

BSA Results

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 (NH₄)₂SO₄ as well as the BSA or Alginate. The colorimetric Nitrate assay is then analyzed with a spectrometer at 540nm. At an end of the experiment each bottle’s contents is analyzed at 600nm to normalize cell concentration.

Method 2: Silver Nanoparticles

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.

Sodium Alginate Results

Thus, each bottle contains a media comprised of 30mM Hepes and 2.5mM (NH₄)₂SO₄, and has a working volume of 35mL. The target concentration of cells is ~7mg/L. After adding various concentrations of BSA/Na-Alginate and sodium ions the bottles are shaken at 250rpm in a 25°C dark room for 3 hours.

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.

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References


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

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