

# Precipitation, Adsorption and Inhibitory Effects of Nano Copper in Activated Sludge

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## ABSTRACT

Bench scale bioreactors were setup to determine the removal and toxicity of nano copper and copper ions in activated sludge biomass. Nano copper was found to be removed more effectively (~ 95%) than copper ions (30 to 70%) from wastewater. The mechanism of removal was found to be aggregation and settling (nano copper) or precipitation (copper ion) rather than biosorption. Most Probable Number (MPN) test showed that 10mg/l ionic copper was toxic to both heterotrophic and ammonia oxidizing bacteria while nano copper showed no significant inhibitory effect. Respirometry studies showed a 55% decrease in respiration rate in the presence of 10mg/l ionic copper. Nano copper again showed no significant decrease in respiration rate. The toxicity to the microorganisms in activated sludge appears to be a function of the fraction and size of copper remaining in solution.

**Keywords:** nanomaterial, nano copper, biosorption, toxicity, bioreactors

## 1 BACKGROUND

“Manufactured Nanomaterial” is an entirely new class of pollutants that wastewater plants need to address due to their unique physical and chemical properties that make nanomaterial attractive for new and better products. By definition nanomaterials are extremely small (1-100nm) and due to their small size they have several different physical/chemical properties, such as conductivity, color, solubility, diffusivity and reactivity can be vastly different than their larger counterparts (1,2). Most nanomaterials have not been studied in detail before and when they are released they may cause harm to humans or the environment. Currently, hundreds of products contain nanomaterials and these products include pesticides, personal care products, food additives, and pharmaceutical agents (3). The market potential for products that contain nanomaterials is expected to exceed \$2.5 trillion by the year 2015. A few studies have been conducted on the health concerns of nanomaterial and research indicates that due to their small size they may

transport through human tissue and bacterial membranes causing cellular, sub-cellular and protein damage (4,5).

Nanocopper materials have wide range of applications as fungicides, cosmetics, printers and electronics (3). However, some recent studies indicate that copper(oxide) nanomaterials have significantly higher potential for cytotoxicity and DNA damage compared to copper ions (6). Therefore, it is vital that the wastewater industry understand and prevent the potential serious problems that copper (oxide) and other nanomaterial can cause to the treatment process.

## 2 OBJECTIVE

The objective of this study is to compare the fate, removal and inhibitory effects of nano copper (oxide) with ionic copper in activated sludge. The impact of functionalization (complexation) of nanocopper as well as the mechanism of nanocopper removal (precipitation, adsorption, sorption to biomass) was also investigated.

## 3 METHODOLOGY

### 3.1 Nano Copper

Nanocopper for this study was obtained from QSI Company (Orange, CA). These particles have a primary size of about 45 nm. Nanocopper stock suspensions (200 mg/l, pH 8.0) were made with and without a complexing agent (ammonium polymethacrylate - Darvan-C). The suspensions were made by adding nano copper to pH 8 DI water then mixed and sonicated for 1 hour.

### 3.2 Copper Removal Study

Two experiments were performed to determine the removal of nano copper and copper ion.  $\text{CuCl}_2$  was used to study the impact of copper ion. Initially, adsorption isotherm studies were performed by spiking 2 to 10 mg/l nanocopper or copper ion in 100 ml activated sludge in 200 ml flasks. Activated sludge was obtained from Orange County

Sanitation District (OCS). After equilibration for 24 hours, the supernatants were analyzed for copper levels. Subsequently, a similar study was performed using activated sludge filtrate (0.45  $\mu\text{m}$ ), to estimate the copper removed by chemical precipitation or physical aggregation. The difference in the amount of copper removed in these two studies yielded the copper removed by sorption to biomass. Nano copper and copper ion were analyzed by ICP-MS (EPA 200.8) Method. Particle size distribution and count of nanomaterials in the samples were evaluated using a Dynamic Light Scattering (DLS) Method.

### 3.3 Most Probable Number (MPN) Test

Inhibitory effects of nanocopper to heterotrophic and ammonia oxidizing bacteria in activated sludge biomass was evaluated through MPN studies. Heterotrophic bacteria required Lauryl Tryptose media with Brilliant Green Bile Broth as the confirmation medium and ammonia oxidizing bacteria required *nitrosomonas* media. A dilution series of  $10^{-6}$  for heterotrophic microorganisms and  $10^{-2}$  for ammonia oxidizing bacteria were made for toxicity evaluation. Triplicate samples were inoculated for each dilution and most probable number was calculated.

### 3.4 Respirometric Test

Specific oxygen uptake rate (OUR) was also conducted using PF-8000 Aerobic/Anaerobic Respirometer from Respirometer System and Application, LLC (Springdale,

AR). Samples spiked with 10 mg/l of nano or ionic copper were placed in serum bottles with an integrated carbon dioxide trap. Pressure changes were monitored continually and a controlled release of necessary oxygen to the bottle as needed while also recording the oxygen uptake in the bottle.

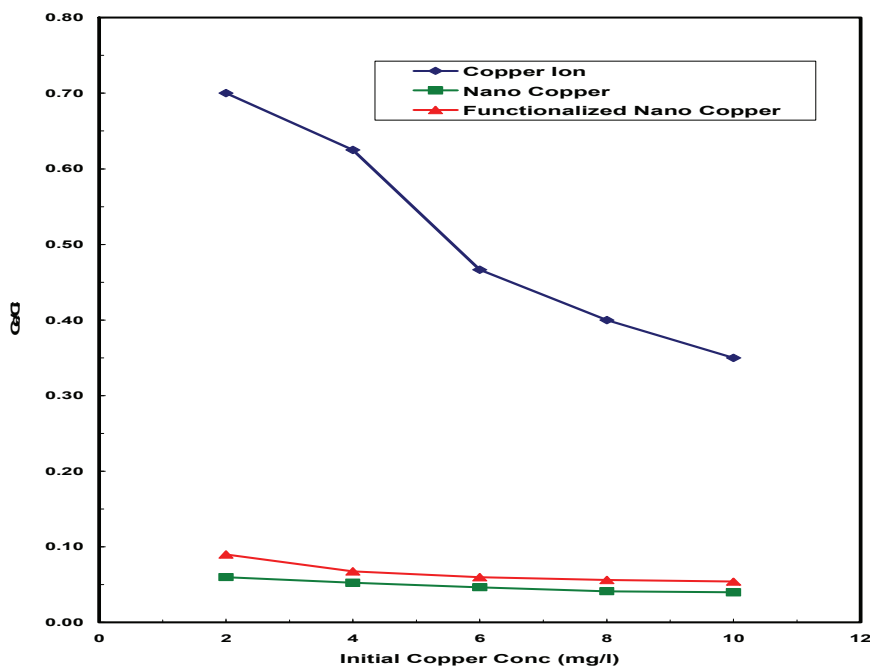
## 4 RESULTS

### 4.1 Copper Removal

Results from the isotherm studies using activated sludge and the activated sludge filtrate indicated that, nanocopper (94 to 96%) was removed more effectively than copper ion (35 to 70 %) (Figure 1). The stabilization agent did not have a significant effect on the nano copper. A second set of tests were performed to determine if the removal of copper is primarily due to adsorption to biomass. Copper ion and nano copper were added to activated sludge filtrate and equilibrated for 24 hours. We found that 75% to 80 % of nanocopper was removed by aggregation/settling and 15 % removed through adsorption to biomass. Nearly 60 % of copper ion was removed by chemical precipitation and 15 % removed through adsorption to biomass.

### 4.2 MPN Results

MPN tests were performed to determine the toxicity of nano copper and ionic copper to heterotrophic bacteria and nitrifying bacteria. Heterotrophic MPN tests spiked with nano copper showed significant growth indicating that nano



**Figure 1.** Initial and final concentration of nano copper and copper ions after equilibration with activated sludge biomass for 24 hours.

copper did not inhibit growth in OCSW wastewater. However, MPN tests spiked with ionic copper showed no bacterial growth suggesting inhibition and possible toxicity. Ammonia oxidizing bacteria showed similar results where nano copper did not vary significantly relative to the control samples and the spiked copper ion samples showed significantly lower growth.

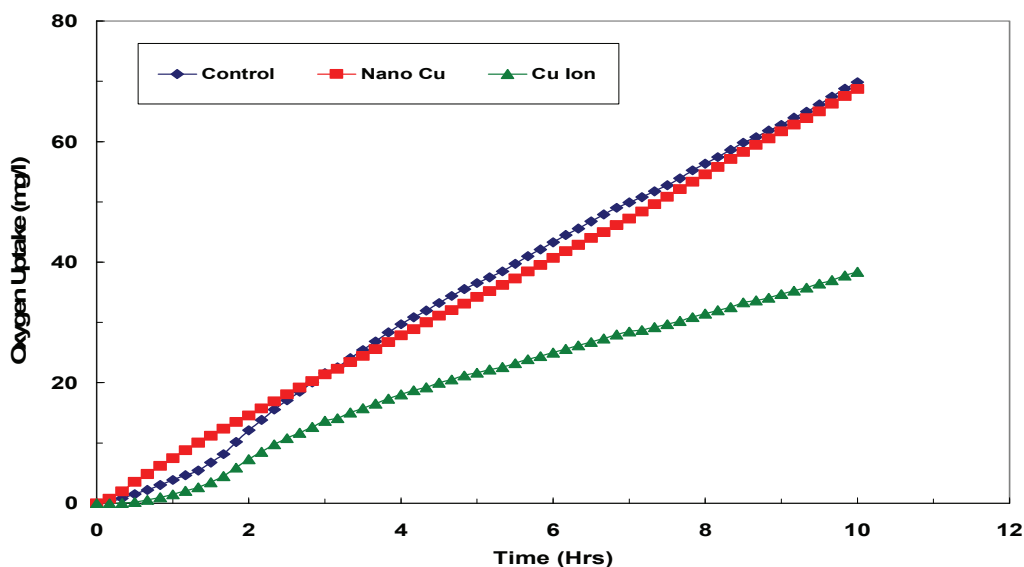
### 4.3 Respirometric Results

The oxygen uptake rate (OUR) was used to estimate the short term toxicity of nanomaterial to microorganisms. OUR tests were consistent with the MPN toxicity test data where the nano copper samples showed no significant effects relative to the control. The copper ion samples were always lower than the control. After 10 hours of incubation the respiration rate for copper ion spiked samples were approximately 55% of the control and nano copper spiked samples (Figure 2).

## 5 DISCUSSION

The differences in the fraction of nanomaterials in suspension may alter the toxic potential of the nanomaterials. In many studies evaluating nanomaterial toxicity, dissolution of nanomaterials appears to play a key role in microbial inhibition. However, evidences also point to some direct toxicity effects caused by nanoscale materials. For example, ionic silver appeared to inhibit the growth of *P. fluorescens* more than nano silver (average size ~ 70 nm) in the presence and absence of humic substances, at pH values 6 to 9 (7). However, at high silver concentration (2000 ppb, the toxicity

effect of nano silver was different than that of ionic silver, suggesting that a nanomaterial specific toxicity may also be involved. The same nanomaterials when exposed to *P. putida* biofilms caused sloughing of the biofilms in the absence of fulvic acid. Fulvic acid facilitated incorporation of nanomaterials into the biofilms. But the presence of nanomaterials did not alter the viability of *P. putida* (8). Also, in a different study, while ionic silver inhibited growth of nitrifying bacteria (100%) more than nano silver (mean size ~ 14 nm; 54% inhibition), nano silver inhibited respiration rate (86%) more than ionic silver (42%) (9). This suggested that a nanomaterial specific toxicity occurred to nitrifying bacteria. In a study relating nanoparticle size to inhibition to nitrifying bacterial growth, nano silver stock containing a larger amount of smaller size (< 5 nm) particles inhibited the growth more than the stock containing more larger size (> 5 nm) nanoparticles (10). However, it is not clear if the smaller nanoparticles directly affected the bacterial growth, or the inhibition was caused by rapid dissolution of the smaller nanoparticles. In a study comparing toxicity of nano copper to bulk (precipitated) copper to *P. subcapitata*, the inhibition to growth of the microalgae was proportional to the amount of bioavailable (based on microbial sensor data) copper in the media, rather than the total or dissolved concentration (11). Nearly 25% of the nano copper and only 0.18 % of bulk copper was bioavailable to the algae tested, and complete inhibition to growth occurred at 6.4 mg/l Cu with nano copper, and 25.6 mg Cu with bulk copper. Investigation of toxicity of nano cerium oxide particles (14 to 29 nm) to *P. subcapitata* indicated that dissolution of cerium oxide or direct effect of the nanoparticles could not explain the inhibitory effects



**Figure 2.** Microbial inhibition measured as cumulative oxygen respiration rate for OCSW activated sludge microorganisms spiked with nano and ionic copper

observed (12). Rather, clustering of nanoparticles around the algae cell was suspected to locally cause a direct or indirect effect. In one study, toxicity of nano copper and nano silver to invertebrate *C. dubia* and *D. pulex* in freshwater could not be explained by dissolution alone, but nano nickel toxicity almost depended on dissolution (13). Also, the toxicity effects (determined from the gene expression profile) of nano copper and nano silver (~ 27 nm; 100 to 1000 µg/l) to zebrafish gill were distinctly different than those exposed to their ionic counter parts, suggesting nanoparticle specific toxicity to these organisms (14). In one study evaluating toxicity of nano copper to human DNA, the cytotoxicity induced by nano copper was about eight times higher than that of ionic copper. Partitioning and mechanisms of toxicity are not currently known. This data appear to indicate that, direct toxicity caused by nanoscale materials occur under some conditions. However, the differences in the test conditions, type and size of nanomaterials used, toxicity and partitioning parameters evaluated render identifying these conditions difficult from these studies.

In our study using OCSD wastewater, nano copper did not inhibit microbial growth (as measured by MPN) or respiration. However, significant inhibition to growth (~100% of heterotrophic and nitrifying bacteria), and respiration (~ 55%) were observed in the presence of copper ions. Nearly 2 mg/l of the 10 mg/l of nano copper and 4.1 mg/l of the 10 mg/l copper ion remained in suspension. Furthermore, the residual copper in the nano copper filtrate was larger in number and size, suggesting that the nano copper in suspension did not completely dissolve. These data suggested that, dissolution of nano copper is required for potential copper toxicity to heterotrophic and nitrifying bacteria in OCSD wastewater. However, since nano copper settled, aggregated, or partitioned with biomass more than the ionic copper, its long term effect on biosolids need to be evaluated. Finally, the residual copper concentration (nano or ionic copper) in all of the partitioning studies were higher than the NTR limit of 8.9 µg/l. Hence, future studies must also focus on removing nanomaterials to meet stringent wastewater discharge limits.

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