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# Bacterial response to a continuous long-term exposure of silver nanoparticles at sub-ppm silver concentrations in a membrane bioreactor activated sludge system

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ABSTRACT

Silver nanoparticles (nanosilver or AgNPs) have excellent antimicrobial properties. Because of their increasing use, there is a concern about the potential impact of AgNPs in wastewater treatment systems. This study investigated the long-term effects of AgNPs (continuous loading for more than 60 days) on membrane bioreactor (MBR) activated sludge performance. At the influent AgNP concentration of 0.10 mg Ag/L, there was no significant difference in effluent water quality or bacterial activities before and after AgNP exposure. Nitrifying bacterial community structure was relatively stable before and after the long-term AgNP loading. Both ammonia-oxidizing bacteria (AOB) *Nitrosomonas* spp. and *Nitrosospira* spp. were present while *Nitrospira* spp. was the dominant nitrite-oxidizing bacterial species throughout this study. Abundance of silver resistance gene *silE* in the MBR, however, increased by 50-fold 41 days after the AgNP exposure, and then decreased with continuous AgNP exposure. The long-term nanosilver exposure did not change the membrane fouling rate although extracellular polymeric substances (EPS) concentration increased significantly after nanosilver dosing. The results suggest that AgNPs at the influent concentrations of 0.10 mg/L and below have almost no impact on activated sludge wastewater treatment performance, as activated sludge can effectively reduce nanosilver toxicity by adsorbing or precipitating AgNPs and silver ions ($Ag^{+}$) released from the dissolution of AgNPs.



## 1. Introduction

Due to a wide range of antimicrobial applications (Meyer et al., 2009), silver nanoparticles (nanosilver or AgNPs) from nanotechnology-enhanced products are released to the environment and discharged to wastewater treatment systems. For instance, AgNPs in the outdoor paintings can be released to reach a peak concentration of 145 μg/L in stormwater runoff (Kaegi et al., 2010). After washing of nanosilver coated consumer products such as clothes and socks, AgNPs

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are eventually discharged into wastewater treatment facilities (Benn and Westerhoff, 2008; Blaser et al., 2008). Such products can leach as much as 1.3 mg of silver per liter in the water (Benn and Westerhoff, 2008). However, after mixing with other wastewater streams, silver concentrations in wastewater treatment plants (WWTPs) are generally low. The estimated concentrations of AgNPs in the effluent and sludge samples of WWTPs are 0.021 μg/L and 1.55 mg/kg, respectively (Gottschalk et al., 2009). The predicted effluent total silver concentrations range from 2 to 18 μg/L (Blaser et al., 2008). However, if WWTPs receive high silver loadings from industrial discharges, the total silver concentration in the influent can be as high as 105 μg/L (Shafer et al., 2009). At high silver concentrations, wastewater treatment process can be adversely impacted. For instance, significant nitrification inhibition has been observed after a 12-h shock loading of nanosilver to reach a maximum concentration of 0.75 mg/L AgNPs in a Modified Ludzacke Ettinger (MLE) activated sludge system (Liang et al., 2010). Nevertheless, such an accident at WWTPs receiving wastewater streams containing high concentrations of silver would be rare, if not impossible.

It is predicted that AgNPs have no effect on the activity and abundance of bacteria involved in wastewater treatment at μg/L or ppb levels (Blaser et al., 2008). Indeed, in a recent short-term (10-days) sequencing batch reactor (SBR) study, a nanosilver dosage of 0.5 mg/L in wastewater per cycle (two cycles per day) did not affect chemical oxygen demand (COD) or $NH_4^+$–N removal (Hou et al., 2012). In another SBR study lasting for 27 d, COD removal was not adversely impacted when AgNPs were dosed at the concentrations from 0.5 to 1.5 mg/L per cycle (around two cycles per day) (Wang et al., 2012). However, probably a more realistic situation in wastewater treatment would be the release of silver including AgNPs continuously into wastewater, but at much lower concentrations. Currently, little is known about the impact of continuous long-term exposure of AgNPs at sub-ppm levels on activated sludge and treatment performance.

Meanwhile, for wastewater treatment and management purposes, there is a desire to establish the same standards and limits for AgNPs as those required by the National Pretreatment Program in the U.S. for emerging chemicals (USEPA, 2004). In the United States, the National Secondary Drinking Water Standard for silver is 0.10 mg/L (USEPA, 1991) to protect human health, but there is no regulation for silver concentration in the sewage entering WWTPs. Considering the effluent of municipal WWTPs that affects the water quality of receiving water, which is often used as a drinking water source, it is desirable to set a similar stringent limit on the levels of silver in the sewage. Although nanosilver can be more toxic than silver ions ($Ag^+$) to nitrifying bacteria that are essential in WWTPs (Choi et al., 2008; Choi and Hu, 2008), it is appropriate to select an influent nanosilver concentration of 0.10 mg/L in wastewater treatment performance evaluation considering the estimated maximum influent silver concentration from industrial discharges (Shafer et al., 2009) while the inhibitory mechanisms of nanosilver are mainly associated with the release of $Ag^+$ ions from AgNPs (Miao et al., 2009; Navarro et al., 2008).

MBR was selected in this study to investigate the potential impacts of long-term nanosilver dosing at low concentrations because it has been increasingly used in WWTPs for high quality wastewater treatment (Rosenberger et al., 2002; Ueda and Hata, 1999). However, membrane fouling is still one of the major issues that limits MBR application. Biofouling due to the formation of biofilms that are embedded in a matrix of extracellular polymeric substances (EPS) of bacteria origin causes irreversible adsorption, pore plugging and cake layer formation on the membrane surface. Nanosilver has strong antimicrobial activities to inhibit bacterial growth and biofilm formation (Choi et al., 2010) on the surface of a membrane. Thus, nanosilver is used in membrane coating or surface modification for preventing membrane biofouling (Kim and Van der Bruggen, 2010; Rana and Matsuura, 2010). For instance, compared with bare polysulfone (PS) membranes, AgNP incorporated PS membranes (with less than 1% wt nanosilver) could significantly reduce biofouling (Koseoglu-Imer et al., 2012). However, it is not clear whether the long-term exposure of attached bacteria on the membrane to AgNPs in the mixed liquor of the MBR would reduce membrane fouling and improve membrane cleaning efficiency. Therefore the objectives of this study were: 1) to determine the impact of continuous long-term nanosilver exposure on activated sludge and wastewater treatment performance, and 2) to evaluate the effect of long-term nanosilver exposure on membrane fouling and control.

## 2. Materials and methods

### 2.1. Nanosilver synthesis and characterization

The nanosilver stock suspension (7.8 μM or 8.60 mg/L) was prepared by chemical reduction methods with minor modifications (Choi et al., 2008; Mulfinger et al., 2007). Briefly, 8.5 mL of 28 mM sodium borohydride ($NaBH_4$, Sigma, St. Louis, MO) solution was added to a 490-mL of 0.6 g/L poly(vinyl alcohol) (Sigma, St. Louis, MO) solution. Then, 2.8 mL of 14 mM silver nitrate ($AgNO_3$, Sigma, St. Louis, MO) solution was added dropwisely (around two drops per second) while the solution was magnetically mixed at approximately 700 rpm. The prepared AgNP suspension was transparent and has a bright yellow color.

The suspension was characterized by a Cary UV–visible spectrophotometer (Varian, CA) for unique surface plasmon absorption band in the presence of nanosilver (Fig. S1). The maximum absorbance of the AgNP suspensions was observed at 400 nm, indicating the appearance of AgNPs (Choi et al., 2008; Petit et al., 1993). Also, the shape of the absorbance curve did not change significantly over two weeks, indicating that the prepared AgNPs was stable under room temperature (Choi and Hu, 2008).

Size distribution of AgNPs was determined by scanning transmission electron microscopy (STEM, JEM-1400 Transmission Electron Microscope, JEOL, Tokyo, Japan) under an acceleration voltage of 120 kV. The histograms of the size distribution were generated from the original STEM images using ImageJ software (http://rsbweb.nih.gov/ij/) (Choi and Hu, 2008) (Fig. S2). The freshly prepared AgNP suspension and the one stored at room temperature for 14 days had an average size of 6.0 ± 2.0 nm ($n = 61$) and 6.4 ± 3.4 nm ($n = 81$),