Journal of Hazardous Materials 341 (2018) 448–456

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research Paper

Contradictory effects of silver nanoparticles on activated sludge wastewater treatment

Zhiya Sheng[a], Joy D. Van Nostrand[b], Jizhong Zhou[b], Yang Liu[a,*]

[a] Department of Civil and Environmental Engineering, University of Alberta, Edmonton, AB, T6G 2W2, Canada
[b] Institute for Environmental Genomics, Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA

HIGHLIGHTS

- Ag-NPs, especially freshly prepared, can have positive effects.
- Ag-NPs can help to maintain microbial community diversity in activated sludge.
- Improved sludge settleability can be important in the positive effects observed.
- The hormesis model may need to be considered for the toxicology of Ag-NPs.

ARTICLE INFO

Article history:
Received 17 April 2017
Received in revised form 22 July 2017
Accepted 24 July 2017
Available online 2 August 2017

Keywords:
Silver nanoparticles (Ag-NPs)
Activated sludge
Microbial diversity
Functional diversity
Geochip
Pyrosequencing

ABSTRACT

Increased amount of nano-silver will be released into domestic and industrial waste streams due to its extensive application. However, great controversy still exists on the effects of silver nanoparticle (Ag-NP) on biological wastewater treatment processes and a toxicology model has not been built yet. Four sequencing batch reactors with activated sludge has been run for over three months with different silver species at a concentration of 1 mg Ag/L in influent. Both freshly prepared Ag-NPs and aged Ag-NPs were tested with released silver ion as control. Results in this study showed that Ag-NPs, especially freshly prepared Ag-NPs, can help to maintain or even increase the diversity of microbial community in activated sludge and the biomass concentration even under long-term treatment. It indicates that the hormesis model need to be considered for the toxicology of Ag-NPs.

1. Introduction

Nano-silver is inevitably released into domestic and industrial waste streams as it is one of the most commonly used nano materials in consumer products [1]. Considerable attention has been paid to the potential adverse effects on biological wastewater treatment system (BWTS) due to the antimicrobial properties of silver nanoparticles (Ag-NPs). A general conclusion can be made from previous research that the effects of Ag-NPs depend on the dose and time period applied as well as the property of Ag-NPs and the system Ag-NPs are applied to. However, great controversy still exists on how each of these parameters affects the impacts of Ag-NPs, and a sophisticated toxicology model has not been built at all. Previous research covers only a tip of the iceberg of all possible combination of these parameters. Not to mention that the mechanisms behind the phenomena are poorly understood.

Higher concentration of Ag-NPs often results in more significant adverse effects [2–5]. Hormetic effects under sublethal concentration have been reported occasionally but stayed as a marginalized concept [6–10]. Properties of Ag-NPs that affect its toxicity include nanoparticle size, shape and coating. Smaller Ag-NPs tend to be more toxic [11–13]. Spherical Ag-NPs and polyvinylpyrrolidone (PVP) coating tend to have weaker bactericidal action [14,15]. However, the effects of shape and coating have not been well-studied yet. Great controversy also exists on if the toxicity of Ag-NPs come from the released Ag^+ ion or the nanoparticle form itself [3,7,14,16–18].

Properties of the BWTS are even more complicated. The effects of Ag-NPs on activated sludge and biofilm in BWTS have been studied previously [19]. Ordered from the most resistant to Ag-NPs to the least, the microbial communities in BWTS include biofilm/activated sludge, planktonic mixed culture and pure culture of single strains

* Corresponding author at: Department of Civil and Environmental Engineering, University of Alberta, Edmonton, AB, T6G 2W2, Canada.
E-mail address: yang.liu@ualberta.ca (Y. Liu).

http://dx.doi.org/10.1016/j.jhazmat.2017.07.051
0304-3894/

[2,9,20,21]. Potential ligands in BWTS can bind with Ag-NPs or released Ag^+ ions and lower the dissolution of Ag-NPs and their bactericidal effects [22–24], although some anions may accelerate Ag-NP dissolution [25]. These ligands range from organic matter such as dissolved organic carbon to inorganic ions such as chloride and sulfide. It has been reported that ammonia oxidizing bacteria (AOB) are more vulnerable towards Ag-NPs treatment, as compared to nitrite oxidizing bacteria (NOB) and organic oxidation heterotrophs [20,26–28]. More recent studies tend to focus on long-term effects of Ag-NPs under conditions mimicking the real-world conditions in BWTS [21,29,30]. Acute inhibition is often observed at the beginning of Ag-NP addition, but the system usually recovers in the long term [4,5]. For instance, in a membrane bioreactor activated sludge system with 0.1 mg/L Ag-NPs in the reactor influent, the silver resistance gene (*silE*) increased at the beginning of the Ag-NPs addition and then decreased to the initial level; and the reactor performance was not significantly affected by Ag-NPs [31]. In a separate study, with the addition of 1 and 5 mg/L Ag-NPs in bioreactors, phosphorus removal decreased and the microbial community changed at the beginning of the study but then stabilized with persistent exposure [30]. The adverse effects of Ag-NPs are minimal especially when sulfidation plays an important role in most of the BWTSs [32–37]. A toxicological model to estimate the effects of Ag-NPs is beginning to take shape. This raises the question: should the hormesis model be considered here?

This study examines the response of the microbial community to a potentially "least-toxic" combination, which is the case in most of our current BWTSs in practical operation: low dose, long-term, spherical Ag-NPs with PVP coating in activated sludge bioreactors fed with synthetic municipal wastewater. No significant effects were seen on pollutants removal. However, interestingly, Ag-NPs helped to maintain the microbial community diversity in the activated sludge. 16S rRNA gene based pyrosequencing was used to monitor the bacterial community and GeoChip was used to directly examine the functional diversity of the microbial community. Properties of the sludge, accumulation of silver species inside the sludge and characteristics of the Ag-NPs were examined to explain this phenomenon.

2. Material and methods

2.1. Reactor setup

Four sequencing batch reactors were operated for over three months. The total volume of the reactors was 1 L and the effective volume was 700 mL. The reactors were run on a 12 hour cycle (5 min of influent filling, 11 h of aeration, 30 min settling, 5 min effluent withdraw and 20 min idle). Hydraulic retention time was 24 h. Sludge was wasted through effluent withdraw by gravity. Solids retention time was monitored but not controlled. Solids retention time (SRT) was 17 days at the steady state for all four reactors. However, after the addition of silver species started, almost no sludge was wasted from the reactor with fresh Ag-NPs added, indicating that SRT was dramatically increased after the addition of fresh Ag-NPs. The reactor feed was prepared according to Alito and Gunsch [5] and contains an average COD of 450 mg/L and ammonia of 40 mg/L with pH adjusted to 7.3 ± 0.5.

2.2. Silver species addition

Self-dispersing silver nanopowder was purchased from SkySpring Nanomaterials, Inc. (Houston, USA). According to the Ag-NP product description, the particle size is less than 15 nm, and the particle composition is 25% silver (99.99% purity) and 75% polyvinylpyrrolidone (PVP), similar to Ag-NPs commonly used in commercial products. Fresh and aged Ag-NP suspensions were examined by transmission electron microscopy (TEM) according to the method described in previous studies [38]. Spherical aggregates of Ag-NPs were observed. The particle size and zeta potential of Ag-NPs were characterized using a Malvern Zetasizer Nano-ZS (Model: ZEN3600, Malvern Instruments Ltd, Worcestershire, UK). Ag^+ ion dissolution was characterized as described in Section 2.5. PVP and silver species addition started after the reactor reached steady state for over two weeks (27 days after start-up). PVP, aged Ag-NPs, fresh Ag-NPs and Ag^+ ion released from fresh Ag-NPs were added to each of the four reactors respectively. Aged and fresh Ag-NPs were added at a concentration of 1 mg Ag/L in influent. This concentration resulted in 0.5 mg Ag/L in the reactor, which falls within a representative range [39] while it was high enough to see significant effects based on previous tests (data not shwon). PVP was added to the control reactor at the concentration of 3 mg/L which is the same as in reactors with Ag-NP addtion. Aged Ag-NPs stock suspension was prepared when reactors were started up and kept at 4 °C in dark and was added into the influent tank and kept under room temperature in dark for one week. Fresh Ag-NPs suspension (3.5 g $Ag\,L^{-1}$) was prepared everyday and 0.1 mL suspension was spiked into the reactor during influent filling in each cycle, producing a concentration equalled to 1 mg Ag/L in influent. For the fourth reactor, to test Ag^+ ion released from fresh Ag-NPs, 0.1 mL of the freshly prepared Ag-NP suspension was added into a dialysis unit (Slide-A-Lyzer™ MINI Dialysis Device, 2 K MWCO, 0.1 mL, Thermo Scientific, USA) and the dialysis unit was put into the reactor during influent filling in each cycle and float in the activated sludge for 12 h before changing to a new one. Equal amount of PVP (3 mg $PVP\,L^{-1}$) was added into the control reactor. The tests of Ag-NP and PVP addition have been performed for over two months. Similar operation of reactors and Ag-NP addition were repeated for several times.

2.3. Reactor performance monitoring

Effluent quality was monitored in terms of COD and ammonium removal using Hach methods 8000 and 10205 [40]. SVI and MLSS were measured according to the standard methods [41]. Reaction kinetics of COD and ammonium removal and nitrate production was also performed using the substrate depletion method [42]. Mixed liquor samples were collected at 15, 30, 35, 60, 90, 120, 150, 180, 210, 240, 300, 480 and 660 min, centrifuged at 3000 g for 10 min at 4 °C, filtered (0.45 μm) and analyzed for COD, ammonium and nitrate. Nitrate was measured using ion chromatography (IC).

2.4. Microbial community analysis

Activated sludge samples were collected in duplicates and genomic DNA was extracted using a Powersoil® DNA Isolation Kit from MO BIO Laboratories, Inc. (Carlsbad, USA). DNA was analyzed with pyrosequencing and GeoChip.

Paired-end sequencing based on the 16S rRNA gene was performed at the Research and Testing Laboratory (Lubbock, TX, USA), using the Illumina MiSeq platform [43]. Primers 28F (5′-GAGTTTGATCNTGGCTCAG-3′) and 519R (5′-GTNTTACNGCGGCKGCTG-3′) were used, which covered V1–V3 hypervariable regions [44]. Chimeras and poor quality sequences were removed from the denoised sequence reads. The remaining sequences were clustered into operational taxonomic units (OTUs) with 0% divergence using USEARCH. Taxonomic information was assigned to OTUs based on a database of high quality sequences derived from the NCBI using a distributed. NET algorithm that utilizes BLASTN+ (Kraken BLAST, www.krakenblast.comwww.krakenblast.com). A principal coordinates analysis (PCoA) of micro-