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Anaerobic toxicity of cationic silver nanoparticles

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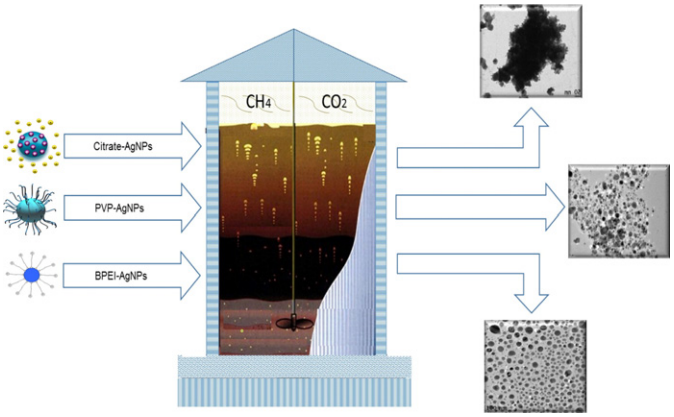
HIGHLIGHTS

- At concentrations -1 the anaerobic decomposition process was not impacted.
- An impact on the microbial community at concentrations -1 were observed.
- At high concentrations (100 mg L^{-1}), the cationic BPEI-AgNPs demonstrated toxicity.
- Toxicity was demonstrated without the presence of oxidative dissolution of silver.
- A one size fits all approach for the evaluation of NPs may not be accurate.

GRAPHICAL ABSTRACT



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ABSTRACT

The microbial toxicity of silver nanoparticles (AgNPs) stabilized with different capping agents was compared to that of Ag^{+} under anaerobic conditions. Three AgNPs were investigated: (1) negatively charged citrate-coated AgNPs (citrate-AgNPs), (2) minimally charged polyvinylpyrrolidone coated AgNPs (PVP-AgNPs) and (3) positively charged branched polyethyleneimine coated AgNPs (BPEI-AgNPs). The AgNPs investigated in this experiment were similar in size (10–15 nm), spherical in shape, but varied in surface charge which ranged from highly negative to highly positive. While, at AgNPs concentrations lower than 5 mg L^{-1}, the anaerobic decomposition process was not influenced by the presence of the nanoparticles, there was an observed impact on the diversity of the microbial community. At elevated concentrations (100 mg L^{-1} as silver), only the cationic BPEI-AgNPs demonstrated toxicity similar in magnitude to that of Ag^{+}. Both citrate and PVP-AgNPs did not exhibit toxicity at the 100 mg L^{-1} as measured by biogas evolution. These findings further indicate the varying modes of action for nanoparticle toxicity and represent one of the few studies that evaluate end-of-life management concerns with regards to the increasing use of nanomaterials in our everyday life. These findings also highlight some of the concerns with a one size fits all approach to the evaluation of environmental health and safety concerns associated with the use of nanoparticles.

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1. Introduction

Nanotechnology has seen a dramatic increase in utilization in environmental, medical, chemical and pharmaceutical industries (Nel

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et al., 2006). Engineered nanoparticles (ENPs) possess unique characteristics, compared to their bulk counterparts, such as the large surface area to volume ratio, high chemical reactivity, unique antimicrobial/fungicidal activity, and biocompatible surface properties (Khaydarov et al., 2009). Most of these properties are directly attributed to the small particle size of nanomaterials and only manifest within a particular size regime (Maynard and Michelson, 2006). These unique properties of materials at the nanoscale raise the need to examine the behavior of these particles in diverse environmental scenarios. Fundamental research on the fate, transport and toxicity of NPs is essential within an effort to determine their impact on natural and engineered environmental systems such as groundwater, soils and sediments, wastewater treatment and municipal solid waste management systems.

In particular, silver which has been well known for its antibacterial properties for centuries has become among the most commercially used nanoparticles (Tolaymat et al., 2010). Currently silver nanoparticles are employed in many consumer products such as textiles, biomedical products, plastics, socks, food storage containers and various cleaning products (Dobias and Bernier-Latmani, 2013). These particles are synthesized using various techniques to produce particles with different chemical and physical characteristics (Iravani et al., 2014). Most of the AgNPs incorporated into consumer products are coated, surface modified and or functionalized to achieve certain properties (Reinhart et al., 2010; Nowack, 2009). Different surface functionalization, obtained by applying different capping agents, was shown to influence the toxicity, aggregation and dissolution of AgNPs (Kvitek et al., 2008; Unrine et al., 2012).

A considerable amount of silver may be released from silver nanoparticle-containing consumer products within just a few washing cycles (Benn and Westerhoff, 2008; Geranio et al., 2009). Once released, depending on the circumstances, AgNPs can end up in a variety of environmental scenarios. It is highly likely that AgNPs travel through municipal sewer lines and reach wastewater treatment plants (WWTPs) and potentially accumulate in the biosolids (Kiser et al., 2012; Shafer et al., 1998; Marambio-Jones and Hoek, 2010).

One of the primary methods of biosolids treatment utilized is anaerobic digestion which relies on active anaerobic bacterial communities to degrade the organics (Donoso-Bravo et al., 2011). Therefore, it is vital to assess the potential impacts AgNPs may have on these bacterial species under anaerobic conditions and also the possible transformations of these particles within these systems. In addition to aggregation, AgNPs may undergo surface changes that influence their behavior. For example, in a system with elevated chlorides and sulfides levels (e.g., wastewater, composter leachate and landfill leachate), the released AgNPs may transform to silver chlorides (AgCl) and silver sulfides (Ag_2S) which are less toxic relative to ionic Ag and metallic AgNPs (Levard et al., 2012; Kim et al., 2010; Gitipour et al., 2013).

It is worthy to mention that the antibacterial properties of AgNPs on microorganisms under aerobic conditions have been significantly studied (Choi et al., 2008). The antibacterial mechanism of silver nanoparticles is linked to a combination of release of Ag^+ by AgNPs oxidative dissolution or specific nanoparticle properties such as Trojan-horse type mechanism and generation of reactive oxygen species (ROS) leading to cell membrane damage (Marambio-Jones and Hoek, 2010; Sotiriou and Pratsinis, 2010). Comparatively, the antibacterial activity of AgNPs under anaerobic conditions has been less studied and understood. According to Kim et al., municipal wastewater treatment plants control the flows of silver between anthropogenic and environmental compartments (Kim et al., 2010). Therefore, the current study aims at investigating the effect of AgNPs on the anaerobic degradation process in simulated environmental systems. The antibacterial impacts of different AgNPs on the digestion process of anaerobic biosolids were studied at various concentrations and compared to that of Ag^+. Based on the observed surface charge dependent impacts, the nanoparticles exhibiting toxicity were further investigated by performing real-time speciation analysis and taxonomical analysis.

2. Experimental section

2.1. Nanoparticles synthesis, purification, and characterization

Three types of AgNPs with different capping agents were utilized, citrate coated AgNPs (citrate-AgNPs), Polyvinylpyrrolidone coated AgNPs (PVP-AgNPs), and branched polyethyleneimine-coated AgNPs (BPEI-AgNPs). The nanomaterials were prepared and purified as described by El Badawy et al. (2010). The hydrodynamic diameter (HDD) and zeta potential (ζ) of the AgNPs were measured using a Zetasizer Nanoseries (Malvern Instruments). Transmission electron microscopy (TEM) was used to verify nanoparticles' size and shape. TEM samples were prepared by depositing a drop of the particular nanoparticle suspension on a carbon coated copper grid. Samples were air-dried at room temperature overnight in a dust-free box. Images were captured using a JEOL-1200 EX TEM (JEOL Inc.) operated at 120 kV. The total Ag concentration of the suspensions was measured using a PerkinElmer AAnalyst 800 atomic absorption spectrometer after performing a microwave acid digestion following EPA method 3015A.

2.2. Anaerobic digesters setup

Anaerobic biosolids were collected from a wastewater treatment plant. The characteristics of the biosolids are presented in Table S1 in Supporting information (SI). In an anaerobic chamber, 30 serum bottles (250 mL total volume) were each filled with 120 mL of biosolids, sealed, covered, and purged with argon for 5 min to initiate anaerobic conditions. The biosolids containing bottles were then placed in a temperature-controlled room at 37 °C and continuously mixed using a bench top shaker. The samples were given two weeks to equilibrate after which each sample received 1 mL cellulose solution (100 g L^{-1}). The experimental setup included three nanomaterial treatments and a positive control containing Ag^+ ($AgNO_3$ was used as the source of Ag^+). Four dosing levels were examined (0.5, 1, 5 and 100 mg L^{-1} as Ag). For consistency, after dosing with the various silver concentrations, de-aired DI-water was added to each serum bottle to achieve a final volume of 150 mL. A negative control composed of biosolids only (no addition of Ag) was also examined. All samples were prepared in triplicates.

2.3. Sampling and analysis

2.3.1. Biogas analysis

Gas sampling occurred immediately after the addition of treatments (time 0) and after 2, 5, 10, 17 and 28 days. The duration of the experiment was chosen to mimic the anaerobic biosolids retention time (25–30 days) at the WWTP from where the sludge was collected. The gas volume generated was measured using an 18 gauge needle and an airtight glass syringe according to US EPA OPPTS method 835.3400 (USEPA, 1998). The gas composition (O_2, CH_4, and CO_2) was evaluated using a gas chromatograph equipped with a thermal conductivity detector (GC/TCD; Agilent 6980N).

2.3.2. Taxonomical analysis

Concurrent to the gas analysis, a 2 mL sludge aliquot was taken from each serum bottle and centrifuged at 5000 rpm for 15 min. After decanting the supernatant, the sample was preserved at −80 °C for taxonomical analysis. Total genomic DNA was extracted from the anaerobic sludge samples using a QIAamp stool DNA mini kit as described by Dowd et al. (2008). Because of the intrinsic uncertainty of the analysis, the data are presented to demonstrate a general trend of the microbial communities and not for their absolute values.

2.3.3. Silver speciation analysis by X-ray absorption spectroscopy (XAS)

To evaluate changes in Ag speciation, X-ray absorption spectroscopy (XAS) was conducted at Sector 10-ID (Segre et al., 2000) of the Advanced Photon Source at Argonne National Laboratory (ANL), Argonne,