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Effects of silver nanoparticles on microbial community structure in activated sludge

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HIGHLIGHTS

- Ag NPs impact microbial communities in intact activated sludge significantly.
- Ag NPs do not impact microbial communities in unsettled activated sludge.
- Activated sludge flocs structure plays roles in the response of bacteria to Ag NPs.

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ABSTRACT

Due to the antimicrobial properties and the widespread use of Ag nanoparticles (NPs) in commercial products, the prevalent Ag NPs in waste streams can decrease the effectiveness of biological wastewater treatments. To determine the effects of Ag NPs on the complex microbial communities present in activated sludge, detailed knowledge of the Ag NPs toxicity on microorganism communities is necessary. Experiments were performed to determine the effects of 1 mg/L Ag NPs on microbial communities in activated sludge. Activated sludge samples with and without gravity settling were compared to evaluate the impact of activated sludge flocs structure on the response of microbial communities to Ag NPs. The effects of Ag NPs on the entire microbial community in activated sludge were analyzed using 16S rRNA gene based polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE). The results suggest that certain microbial species in the intact activated sludge were highly sensitive to Ag NPs treatment, although no reduction in cell culturability was detected through heterotrophic plate counts (HPCs) during the 24 h Ag NPs treatment. Conversely, one log unit reduction in the HPCs with no microbial community structure changes was observed for unsettled activated sludge flocs (intact activated sludge treated by 3 h gravity-settling) after 24 h Ag NPs treatment. This study strongly suggests that Ag NPs can impact the activated sludge microbial community and cell culturability depending on the physical structure of the activated sludge flocs, the spatial distribution of microorganisms in activated sludge flocs, and the community structures in the activated sludge.

1. Introduction

Nanotechnology, emerging in the 1980s, has grown in the 21st century to have the potential to profoundly affect human life. Due to the large surface areas and quantum effects of very small particles, nanoparticles (1 to 100 nm in size) possess unique physical and chemical properties not seen in their macroscopic counterparts. The enhanced strength, durability, flexibility, and performance associated with nanomaterials have been exploited in a multitude of applications, including consumer products, alternative energy, and medicinal uses. Despite bright outlooks for the future of nanotechnology, concerns are expressed over the burgeoning availability of such novel and reactive particles to humans and the environment, particularly the natural aquatic environment (Ju-Nam and Lead, 2008). For instance, with the wide use of NPs in commercial products, it is inevitable that NPs will be released to waste streams. A recent study on the fate of NPs in wastewater treatment systems showed that most NPs are retained in biological wastewater treatment systems (Kiser et al., 2009). Activated sludge, a suspended microbial aggregate, is frequently applied to wastewater to remove organic compounds and nutrients (nitrogen and phosphorus) through the metabolic reactions of microorganisms. Activated sludge is a collection of biological flocs that consist of microorganisms, extracellular biopolymers, and organic and inorganic compounds (De Clercq et al., 2004; Metcalf and Eddy, 2003). Because many nanoparticles are designed to inhibit or prevent biological activity, nanoparticles retained in the activated sludge flocs could decrease the effectiveness of contaminant removal.

The antimicrobial properties of engineered nanoparticles have been investigated in recent studies (Cho et al., 2005; Kang et al., 2007; Lyon et al., 2008; Morones et al., 2005). Among different engineered nanoparticles, the antimicrobial activities of Ag NPs have been studied the most widely because they are commonly used to disinfect appliances in the home, in medical institutions, and in food industries (Konopka et al., 2009; Silvestry-Rodriguez et al., 2008). Various antimicrobial mechanisms of Ag NPs have been examined.

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For instance, Choi et al. (2008) showed that, due to their small size, Ag NPs can pass through cell membranes and accumulate in the cell causing cell malfunction. Other studies showed that Ag NPs attached to the cell membrane can change membrane permeability and cause cell death (Lok et al., 2006; Morones et al., 2005; Sondi and Salopek-Sondi, 2004). Some researchers have suggested that Ag NPs might weaken cell membranes by damaging enzymes (Morones et al., 2005; Matsumura et al., 2003). Gao et al. (2011) and Dimkpa et al. (2011) found that Ag NPs did not damage the cell membrane but affected electron transport. Another possible Ag NPs antimicrobial mechanism is DNA damage that prevents cell replication (Berger, 2007). Release of Ag^+ can also contribute to the antimicrobial effect of Ag NPs (Morones et al., 2005). The overall negative charge of bacterial extracellular polymeric substances (EPS) repels Ag NPs (Luongo and Zhang, 2010; Sheng and Liu, 2011) which are also negatively charged and thus protects bacteria from Ag NPs toxicity. EPS can also quench reactive oxygen species (ROS) generated by Ag NPs treatments or EPS may bind Ag^+ released from Ag NPs and reduce the extent of cellular contact. However, EPS protection can be circumvented at high concentrations of Ag NPs (Dimkpa et al., 2011).

The efficiency with which Ag NPs inhibit the growth of microorganism has been evaluated in several studies. For instance, Choi et al. (2008) showed that Ag NPs at a concentration of 1 mg/L can inhibit more than 80% of the growth of autotrophic nitrifying organisms. In the same study, Ag NPs had the highest potential among all the Ag species tested (including Ag NPs, Ag^+ ions, and AgCl colloids) to inhibit the growth of nitrifying bacteria. Choi and Hu (2008) showed that the effect of Ag NPs on bacteria growth was size-dependent. Ag NPs of less than 5 nm in size were more toxic to bacteria than larger Ag NPs. Similar results were obtained by Elechiguerra et al. (2005) who found that the average size of Ag NPs penetrating an *Escherichia coli* membrane was about 5 nm.

Previous research has concentrated on the effect of Ag NPs on pure cultured microorganisms, such as *E. coli* cultivated under laboratory conditions (Dror-Ehre et al., 2009; Elechiguerra et al., 2005; Sondi and Salopek-Sondi, 2004; Yoon et al., 2007). The Web of Science database contains few studies that investigated the impact of Ag NPs or other nanomaterials on complex microorganism communities (Bradford et al., 2009; Fabrega et al., 2011; Nogueira et al., 2012; Sheng and Liu, 2011). Sheng and Liu (2011) found that original wastewater biofilms were resistant to Ag NPs treatment but became more sensitive after soluble EPS was removed from the biofilms. Bradford et al. (2009) reported that the microbial community diversity in estuarine sediments was only slightly impacted by Ag NPs treatment. Fabrega et al. (2011) found that Ag NPs had significant adverse effects on microbial communities in natural marine biofilms. These conflicting results might be due to different environmental factors or different microbial community structures. Several kinds of nanoparticles have detrimental effects on microbial activities and survivability in wastewater activated sludge, for example, the toxicity to microbes of ZnO NPs (Liu et al., 2011), carbon nanotubes (CNTs) (Goyal et al., 2010; Luongo and Zhang, 2010), TiO_2 NPs, and SiO_2 NPs (Zheng et al., 2011, 2012) has been documented. Microbial communities in activated sludge were very sensitive to CNTs treatment and long term TiO_2 NPs and SiO_2 NPs treatments. Consistent with Sheng and Liu's (2011) results, Luongo and Zhang's (2010) results suggested that EPS can protect microbial communities from the toxicity of CNTs.

The overall objective of the present study was to investigate the impact of Ag NPs on the microbial communities in wastewater activated sludge. Further, the physical structures of activated sludge flocs are essential for their biological treatment efficiency; these structures can be evaluated using flocs settleability. Activated sludge flocs with good settleability are usually large in size, while flocs with poor settleability are usually small (pinpoint flocs) (Andreadakis, 1993; Grijspeerdt and Verstraete, 1997; Jin et al., 2003). Since mass transfer into the microbial flocs is driven by diffusion, the physical structure of activated sludge flocs may be a critical factor for the diffusion and transportation of Ag NPs inside the flocs. The spatial distribution of the microbes on activated sludge flocs is also important to bacterial activities. It is possible that bacteria on the surface of activated sludge flocs are more exposed to Ag NPs. In addition, the toxicity of Ag NPs may be strain-dependent. Community structures of microorganisms in activated sludge may impact the response of bacteria to Ag NPs. To our knowledge, no study has been performed to evaluate the impact of Ag NPs on the microbial communities in wastewater activated sludge, particularly, the impact of activated sludge floc structures on the microbial response to Ag NPs. We hypothesize that the impact of Ag NPs on activated sludge microbial community structures depends on the physical structure of the activated sludge flocs, the spatial distribution of microorganisms in activated sludge flocs, and the microbial community structures in activated sludge. Molecular biology techniques were employed, and 16S rRNA gene based polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) was applied to analyze the microbial community shift after Ag NPs treatment of activated sludge.

2. Materials and methods

2.1. Activated sludge samples

4 L activated sludge samples were collected into a cylinder with a height of 25 cm and a diameter of 15 cm in February, 2011 (temperature of the collection site was 15 °C) from the Gold Bar Wastewater Treatment Plant located in Edmonton, Alberta, Canada. Samples were collected right before each experiment and stored in an ice box during the 30 min transport to the laboratory. Samples were processed at 15 °C (to simulate a similar environment to that in the activated sludge system in Gold bar Wastewater Treatment Plant, Edmonton, Alberta) within 12 h of arrival at the laboratory. The pH of the wastewater was 6.9 ± 0.1 and the ionic strength was 15.5 mM. Activated sludge samples were allowed to settle by gravity to separate good settleability flocs and poor settleability flocs; the intact activated sludge (samples at time 0 h) and unsettled activated sludge (intact activated sludge treated by 3 h gravity-settling) were removed and used for Ag NPs toxicity experiments. Light microscopy was applied to photograph activated sludge samples. A drop (5 μL) of the sludge sample was placed on the microscope slide, covered with a cover glass, and observed at 10× objective under a light microscope. The floc size distribution was determined based on the light microscopy images. The surface structure of microorganisms in the intact sludge and in the unsettled sludge with and without Ag NPs treatment was examined with a scanning electron microscope (SEM) following procedures reported previously (Sheng and Liu, 2011). In order to further examine the presence and distribution of EPS in the activated sludge flocs, EPS and bacterial cells in activated sludge samples were stained using concanavalin A (ConA) conjugated with Taxes Red (Ni et al., 2009) and SYTO 9 green fluorescent nucleic acid dye, respectively, to facilitate fluorescence microscopic observations (20× objective). Each experiment was performed in triplicate.

2.2. Preparation and characterization of Ag NPs suspensions

Self-dispersing silver nanopowder was purchased from SkySpring Nanomaterials, Inc. (Houston, USA). The Ag NPs product description stipulated a particle size of less than 15 nm, and a particle composition of 10% silver (99.99% purity) and 90% polyvinylpyrrolidone (PVP), similar to Ag NPs commonly used in commercial products (Brar et al., 2010). Ag NPs stock suspension of 100 mg/L were prepared by dispersion in ultrapure water, mixing by vortex at maximum speed, and sonication for 2 h. To investigate the impact of wastewater environment on the size stability of Ag NPs, filtered wastewater (filtered through 0.22 μm membranes to remove particulate materials) was used to