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Comparison of nanosilver removal by flocculent and granular sludge and short- and long-term inhibition impacts

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ABSTRACT

The growing production and application of silver nanoparticles (Ag NPs) increases the chance of these particles entering wastewater treatment plants and interacting with activated sludge. In this paper, the removal of Ag NPs by flocculent and granular sludge was studied, and the short- and long-term inhibitory impacts of Ag NPs on the sludge were compared. Results showed that both forms of sludge contributed to removal of Ag NPs with 30–58% and 2.5–9.4% removal by the flocculent sludge and the granular sludge, respectively, at Ag NP dosage of 1–8 mg/L. Exposure to Ag NPs had greater inhibitive effects on the flocculent sludge than the granular sludge. Short-term (12 h) exposure to Ag NPs at 1, 10, 50 and 100 mg/L reduced the ammonia oxidizing rate of the flocculent sludge by 21.0–24.9%, while no inhibition was found for the granular sludge; the oxygen uptake rate of the flocculent sludge was inhibited at Ag NP concentrations as low as 1 mg/L, while that of the granular sludge was only affected at much higher Ag NP concentrations (50 and 100 mg/L). The denitrification rate, however, was not inhibited for either sludge. After long-term (22 day) exposure to 5 and 50 mg/L of Ag NPs, flocculent sludge was significantly inhibited on ammonia oxidizing rate, denitrification rate and oxygen uptake rate, but the microbial activity of granular sludge was not inhibited. Exposure to Ag NPs resulted in oxidative stress and damage of bacterial cell integrity for both flocculent and granular sludge as was determined by generation of reactive oxygen species and release of lactate dehydrogenase (LDH). The toxic effect of Ag NPs on sludge was mediated via both ROS-dependent and ROS-independent pathways, and both small (<10 nm) and large (>10 nm) Ag NPs contributed to it. Overall, granular sludge demonstrated stronger resistance to the toxicity of Ag NPs than flocculent sludge, while flocculent sludge was more efficient in removing Ag NPs.

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1. Introduction

Silver nanoparticles (Ag NPs) are widely used as a catalyst or antimicrobial coatings in many applications including solar energy, consumer products and water sterilization (Nel et al., 2006). With increasing production and application of Ag NPs, there would be increasing release of them into wastewater treatment plants (WWTPs). WWTPs are important barriers to prevent nanoparticles from entering the natural environment (Nowack and Bucheli, 2007). A considerable amount of nanoparticles could be removed via aggregation, settling, precipitation, biosorption, or other biomass mediated processes in WWTPs (Kiser et al., 2009; Liang et al., 2010). Biomass has a strong affinity to nanoparticles and plays an important role in their removal (Kiser et al., 2009; Park et al., 2013). On the other hand, due to the antimicrobial properties of Ag NPs, interaction of activated sludge with Ag NPs may also influence the performance of WWTPs.

Several studies have reported the interactions of engineered nanoparticles (NPs) with flocculent activated sludge. Kiser et al. (2010) studied the removal of eight types of NPs using activated sludge as the sorbents and found that 97% of nonfunctionalized Ag NPs was removed by aggregation and sedimentation. Liang et al. (2010) investigated the bacterial response to a shock load of Ag NPs (1 mg/L, 12 h) in an activated sludge treatment system, and found significant inhibition of nitrification (46.5%) after more than one-month operation. Zheng et al. (2011) studied the effects of ZnO NPs on the biological removal of nitrogen and phosphorus from wastewater. Their results showed that exposure to 10 and 50 mg/L of ZnO NPs lowered nitrogen removal efficiency from 81.5% to 75.6% and 70.8%, respectively. The lower nitrogen removal was attributed to the release of zinc ions and the increased production of reactive oxygen species (ROS).

The antibacterial mechanisms of Ag NPs have been extensively studied with pure cultures of model bacteria such as *Escherichia coli* in the planktonic form (Choi et al., 2008; Fabrega et al., 2009). Ag NPs could impose toxicity through several mechanisms: Ag NPs attach to cell membranes and cause changes in membrane permeability; small Ag NPs (<10 nm) and Ag^+ released may enter the bacterial cell and cause cellular enzyme deactivation, membrane permeability disruption, and accumulation of intracellular radicals (Carlson et al., 2008; Jiang et al., 2008), resulting in microbial growth inhibition, cell lysis and death.

Activated sludge generally exists in two different forms, suspended form such as flocculent sludge and aggregated form such as biofilm and granular sludge. Aggregated sludge generally has a much more complex and heterogeneous structure than flocculent sludge (Liu and Tay, 2004). Aerobic granular sludge is a special existence of microbial aggregation, which forms through self-aggregation under specific conditions and often appears in sequence batch reactors (SBRs) (Liu and Tay, 2004; Quan et al., 2012). Granular sludge can be considered as a special case of biofilm with a three-dimensional and more complex structure, in which microbes are attached to each other and embedded in an extracellular matrix, with different functional microbial populations located in different spaces (Adav et al., 2008). Generally, aerobic heterotrophic microorganisms and some autotrophic microorganisms such as nitrifying bacteria reside in the outer layers of granular sludge, while facultative or anaerobic bacteria such as denitrifiers exist in the inner parts. Sludge in different form may respond differently to the nanoparticles. Biofilm was reported to be more tolerant to Ag NPs than planktonic sludge due to the protective function of extracellular polymeric substances (EPS) and the interactions within the microbial community (Sheng and Liu, 2011). However, interactions of Ag NPs with granular sludge and their inhibitory impacts have not been investigated.

In this study, the removal of Ag NPs by two different types of sludge (granular sludge and flocculent sludge) was compared and the short- and long-term inhibition impacts of Ag NPs on these two sludge types were investigated. Inhibition of Ag NPs on sludge microbial activity was assessed in terms of ammonia oxidizing rate, denitrification rate and specific oxygen uptake rate (SOUR). ROS accumulation and Lactate dehydrogenase (LDH) release were also measured in order to reveal toxicological mechanisms. This study will help understand the transport, fate, and impact of Ag NPs in biological wastewater treatment systems and establish a proper strategy to control these nanoparticles.

2. Materials and methods

2.1. Sources of Ag NPs and activated sludge

Powdered silver nanoparticles with purity of 99.9% and an average diameter of 50 nm were purchased from Beijing Nanopowder Company (Beijing, China). A 100 mg/L Ag NPs stock solution was prepared by dispersing 100 mg of Ag NPs into 1 L of Milli-Q water, followed by sonication for 1 h (25 °C, 250 W, 40 kHz). The particle size distribution of Ag NPs was determined by dynamic light scattering (DLS) (Dynapro Titan TC, Wyatt Technology, USA), which showed an average diameter of 20 nm. The Ag NPs stock solution was sonicated for a 20 min prior to each dosing experiment.

The flocculent activated sludge was collected from the sedimentation tank of a municipal wastewater treatment plant in Beijing, China. The aerobic granular sludge seed was withdrawn from a lab bioreactor which had been culturing aerobic granular sludge for more than one year. Both flocculent and aerobic granular sludge (6 g Mixed Liquor Volatile Suspended Solids (MLVSS)/L) were first acclimated to a synthetic wastewater in two parent SBRs for about two months until achieving a stable performance. The synthetic water contained the following (mg/L): glucose 1060, NH_4Cl 90, $K_2HPO_4 \cdot 3H_2O$ 400, $KH_2PO_4 \cdot 2H_2O$ 340, $CaCl_2 \cdot 2H_2O$ 10, $MgSO_4$ 50 and NaCl 50. To maintain the original sludge types during sludge acclimation, the two SBRs had the same working volume (1.5 L) but different dimensions. The SBR for granular sludge acclimation was a column of 80 cm in height and 5 cm in diameter, while the SBR for flocculent sludge acclimation was 20 cm in height and 10 cm in diameter. The SBRs were operated in daily 6-h cycles consisting of 5 min feeding, 255 min aeration, 90 min settling and 10 min decanting periods. The volume exchange ratio was 50%. Fine air bubbles were supplied at a flow rate of 1.5–2 L/min through a dispenser