Removal characteristics of engineered nanoparticles by activated sludge

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abstract

Environmental release of engineered nanoparticles (NPs) has been on the rise due to the increased use of NPs in commercial products. In addition, the fate of NPs in sewage treatment processes may play an important role in determining the environmental release pathway of NPs. In this study, we investigated the removal of engineered NPs (AgNPs, TiO$_2$NPs, and SiO$_2$NPs) using activated sludge by evaluating the effects of several important factors of the NPs, including physicochemical properties, contact time between NPs and activated sludge, aquatic chemistry of sewage, and the presence of extracellular polymeric substances (EPS) in the activated sludge. For all three types of NPs tested, a considerable amount of NPs were removed after exposure to activated sludge in a time-dependent manner; nevertheless, the removal efficiencies depended on the type of NPs and seemed to be affected by the NP stability relative to the hydrodynamic diameter (HDD) and zeta potential. In addition, the presences of both ionic compounds and EPS significantly enhanced the NP removal efficiency, indicating that the instability of the NPs resulting from the ionic strength in sewage and entrapment of NP by EPS played an important role in NP removal by activated sludge. These results suggest that the removal efficiencies can be affected by the operating conditions of the activated sludge process and the conditions of the activated sludge; therefore, these factors should be considered when developing approaches to sufficiently remove NPs from sewage treatment plants.

1. Introduction

Recently, commercial use of nanoparticles (NPs) in chemical products has dramatically increased with the development of nanotechnology due to their unique properties; extremely high surface area and reactivity (Nel et al., 2006; Zhang et al., 2011). According to a recent survey, NPs are widely used even in daily life products, including personal care, clothing, and cosmetics. In addition, the number of these products in the commercial market has significantly increased in recent years (Project on Emerging Nanotechnologies, 2011). Therefore, the potential risk of releasing NPs into the natural environment threatens human health and ecosystems (Handy and Shaw, 2007; Bae et al., 2010; Akaighe et al., 2011; Dallas et al., 2011), suggesting that the fate and transport of NPs should be investigated thoroughly to minimize their negative environmental risk.

Sewage treatment processes may play an important role in determining the environmental pathways and disposal of NPs that are incorporated into different products (Jarvie et al., 2009; Brar et al., 2010). Typical domestic sewage treatment plants (STPs) mostly operate conventional or modified activated sludge processes and several previous studies have confirmed that a

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considerable amount of the NPs released into the activated sludge process can be absorbed by the activated sludge, resulting in a reduction of NPs in the effluent and an accumulation of biomass (Kiser et al., 2009; Liang et al., 2010; Kaegi et al., 2011). Even though several studies reported the fate of NPs in wastewater treatment plants, the removal characteristics of NPs by activated sludge are not well investigated. The particle instability by wastewater in the absence of activated sludge was just examined (Limbach et al., 2008; Jarvie et al., 2009) or the simple fate of nanoparticles in wastewater treatment plants (Kiser et al., 2009; Kaegi et al., 2011; Wang et al., 2012) was reported.

The aim of this study was to investigate the removal characteristics of engineered NP by activated sludge. Silver (Ag), titanium dioxide (TiO₂), and silica dioxide (SiO₂) NPs were selected as model NPs due to their high potential for environmental release, high quantities of these NPs in production and their wide spread use in commercial products (Bradford et al., 2009; Project on Emerging Nanotechnologies, 2011). The goal of this study was to better understand the removal characteristics, effects of physicochemical characteristics of NPs, contact time related to HRT, aquatic chemistry, and EPS on NP removal.

2. Materials and methods

2.1. Preparation and characterization of nanoparticle suspensions

Stock suspensions of AgNPs coated by citric acid were purchased from ABC Nanotech Co. (STU206011, Korea). The stock suspension containing 20 wt.% AgNPs was serially diluted in DI water (Millipore, France) to the desired concentration. Suspensions of TiO₂NPs and SiO₂NPs, TiO₂NPs and SiO₂NPs were respectively purchased from Evonik Co. (P25, Germany) and Sukgyung AT Co. (Korea) and were suspended in DI water by mixing at 200 rpm for 24 h. The NP sizes in the prepared suspension were determined by transmission electron microscopy (TEM, JEM-3010, JEOL, Japan) and electron light scattering spectrophotometry (ELS-8000, Otsuka, Japan). ELS was also used to evaluate the zeta potential (ζ), which is the magnitude of the electrical charge at the double layer. The pH of the NP suspension was measured using a pH meter F-54BW (Horiba, Japan). The pH of all NP suspensions was 7.0 ± 0.5, which would unlikely change the physical or chemical interactions between the activated sludge and NPs. Thus, no additional pH adjustments were performed.

2.2. Synthetic wastewater

Synthetic wastewater was prepared using glucose (Sigma–Aldrich, USA) with a target chemical oxygen demand (COD) concentration of 200 mg L⁻¹. The synthetic wastewater also contained micronutrients including 10 mg L⁻¹ yeast extract, 10 mg L⁻¹ bactopeptone, 50 mg L⁻¹ (NH₄)₂SO₄, 30 mg L⁻¹ KH₂PO₄, 30 mg L⁻¹ KH₂PO₄, 1.8 mg L⁻¹ MgSO₄, 0.04 mg L⁻¹ FeCl₃, 1.4 mg L⁻¹ NaCl, 0.04 mg L⁻¹ CaCl₂, 0.48 mg L⁻¹ CoCl₂, and 30 mg L⁻¹ NaHCO₃, which was consistent with the composition of synthetic wastewater previously reported (Yeon et al., 2008) with a slight modification. The ionic strength of the synthetic wastewater was ca. 3 mM.

2.3. Activated sludge preparation and EPS removal

The activated sludge was prepared from a lab-scale continuous bioreactor with a total reactor volume of 3.5 L. The bioreactor with ca. 3.0 L of mixed liquor at the mixed liquor suspended solids (MLSS) concentration of ca. 3000 mg L⁻¹ was operated at a hydraulic retention time (HRT) of 8 h and a solids retention time (SRT) of 10 d. Fresh activated sludge obtained from the aeration tank of the Recopark sewage treatment plants (Gimpo, Korea), was acclimated by feeding the synthetic wastewater for more than two months in the lab-scale continuous bioreactor. An aliquot (ca. 20 mL) of the acclimated activated sludge was collected from the bioreactor and centrifuged at 1000g for 10 min to separate activated sludge from the synthetic wastewater. The pellet was resuspended in DI water or synthetic wastewater, and then the adsorption experiment was conducted.

The EPS of the sampled activated sludge was extracted using ethylenediaminetetraacetate (EDTA) as described previously (Cao et al., 2011). Briefly, the sludge was exposed to 25 mM of EDTA containing a 0.9% NaCl solution (pH 7) at 4 °C for 3 h. The microorganisms and EPS of the sludge were then separated by centrifugation three times at 5000g with DI water. The polysaccharide concentration of the EPS solution was quantified using the phenol/sulfuric method as described elsewhere (Dubois et al., 1956). Two milliliters of the extracted EPS solution, 0.025 mL of phenol, and 2.5 mL of sulfuric acid were mixed for 30 min, and then the absorbance of the solution was measured at 490 nm using an Agilent 8453 UV/vis spectrophotometer (Agilent, Germany).

2.4. Batch adsorption experimental with activated sludge

Approximately 3000 mg L⁻¹ MLSS of the activated sludge was exposed to the desired concentration of the NP suspensions at 100 rpm and 25 °C. After the indicated contact time, the activated sludge was centrifuged at 5000g for 20 min, and the supernatant was withdrawn and the NP concentration quantified. The concentration of NPs was quantified by measuring the elements (Ag, Ti, and Si) using inductively coupled plasma (ICP) spectrometry and by taking into account the effluent concentration of the sewage treatment process. For Ag and Ti quantification, and ICP-Mass Spectrometer (Elan 6100, Perkin–Elmer Sciem, Canada) was used. The Si content was measured using an ICP-Optical Emission Spectrometer (ICP-730 ES, Varian, Australia). The detection limits of both ICPs were approximately 1 µg L⁻¹. In addition, the percent removal efficiency was calculated using the following equation:

$$\text{NP removal(%) } = \frac{C_{\text{influent}} - C_{\text{effluent}}}{C_{\text{influent}}} \times 100(%)$$

where $C_{\text{influent}}$ is the NP concentration in the effluent after the contact time and $C_{\text{effluent}}$ is the initial NP concentration in the influent.

All experiments were conducted under dark conditions to prevent the photocatalytic activity of TiO₂. The results are presented as the means and standard deviations from duplicate experiments.

2.5. TEM analysis of nanoparticles in activated sludge

All chemicals used in the TEM analysis were purchased from Electron Microscopy Sciences Co. (Hatfield, USA). The TEM specimens obtained from the sludge exposed to the NPs were prepared as described previously (Lee et al., 2008). The sludge was concentrated by centrifugation at 2500g for 2 min to remove the supernatant. The collected sludge was fixed in 2% glutaraldehyde and 0.05 M sodium cacodylate buffer (pH 7.2) for 2 h and washed three times with 0.05 M sodium cacodylate buffer. The samples were then exposed to 1% osmium tetroxide in 0.05 M sodium cacodylate buffer for 2 h and stained with 2% uranyl acetate for 18 h. Next, the samples were infiltrated in propylene oxide, embedded in Spurr’s resin, and polymerized at 70 °C for 24 h. The prepared polymerized blocks were sectioned using an ultramicrotome (MT-X, RMC, USA) and TEM (JEM-3010, JEOL, Japan) analysis was conducted.
3. Results and discussion

3.1. Characteristics of engineered nanoparticles

Table 1 lists the size characteristics (TEM size and HDD) and zeta potential of the three engineered NPs employed in this study. The TEM size was determined based on the TEM images. As shown in Table 1, the size of three NPs widely varied and were 40, 17, 48 nm for the AgNPs, TiO\textsubscript{2} NPs, SiO\textsubscript{2} NPs, respectively in terms of TEM size and 62, 202, and 171 nm for AgNPs, TiO\textsubscript{2} NPs, SiO\textsubscript{2} NPs, respectively in terms of HDD. The zeta potential of the three NPs varied from \(\pm 21\) mV to \(\pm 53\) mV. Due to the agglomeration and/or aggregation of NPs, the sizes determined by HDD were much larger than those determined by TEM. Intriguingly, the TiO\textsubscript{2} NPs were the smallest by TEM analysis and the highest by HDD analysis (Table 1). This was likely due to particle agglomeration or aggregation. AgNPs which were coated by citric acid, in which improves their stability in DI water and resulted in a smaller HDD and higher zeta potential than the other two NPs.

3.2. Effect of contact time on nanoparticle removal by activated sludge

Fig. 1 shows the removal of three engineered NPs in DI water by activated sludge with time, which is expressed as the remaining concentration (a) and the % removal (b) when the initial concentration of the three NPs employed was 10 mg L\textsuperscript{-1} (in the case of AgNPs, the NPs concentration was 1 mg L\textsuperscript{-1}). As shown in Fig. 1, considerable removal of all three NPs by the activated sludge was observed in a time-dependent manner. More than 90% of the three engineered NPs were removed within 24 h. However, the removal varied depending upon the types of NPs and their initial concentrations. For example, 95% of the TiO\textsubscript{2}NP and SiO\textsubscript{2}NP were easily removed within 1 h. In contrast, only about 50% were removed for the 10 mg L\textsuperscript{-1} of AgNPs, although this value steadily increased up to about 90% after 24 h. One explanation for this difference may be due to the difference in particle stability related to the HDD and zeta potential (Table 1). In addition, when 1 mg L\textsuperscript{-1} of AgNPs was employed, approximately 99% of the NPs were removed by the activated sludge within 24 h, indicating the importance of the NPs to activated sludge ratio. Even though low concentrations of all NPs were detected in the effluent after 24 h (inset of Fig. 1a), the concentration was consistently reduced with contact time. This

![Fig. 1. NP removal by activated sludge as a function of contact time. (a) NP concentrations in the effluent, (b) percent removal of NPs (Deionized (DI) water condition, 3000 mg L\textsuperscript{-1} MLSS, 8 h, 100 rpm, 25 °C).](image1)

![Fig. 2. NP removal by activated sludge in synthetic wastewater versus DI water (3000 mg L\textsuperscript{-1} MLSS, 100 rpm, 25 °C).](image2)

![Fig. 3. Change in the average size of NPs (measured as HDD) with contact time in synthetic wastewater (10 mg L\textsuperscript{-1}, 100 rpm, 25 °C).](image3)
result suggests that HRT, which is directly related to the contact time between the NPs and activated sludge, can be a determining parameter for the removal efficiency.

3.3. Effect of ionic compounds on nanoparticle removal by activated sludge

Fig. 2 shows the removal of NPs in synthetic wastewater (ca. 3 mM ionic strength) by the activated sludge at initial NPs concentrations of 10 mg L\(^{-1}\) (in the case of AgNPs, the NPs concentration was 1 mg L\(^{-1}\) of NPs,) and the contact time was fixed at 8 h. For comparison purposes, the results of the DI experiments (Fig. 1) were included in Fig. 2 (empty bar). As shown Fig. 2, removal of AgNPs and TiO\(_2\)NPs by the activated sludge in the synthetic wastewater was much higher than removal in DI water, while the removal of SiO\(_2\)NPs by the activated sludge was reduced in the synthetic wastewater. For example, in the synthetic wastewater (10 mg L\(^{-1}\) NP exposure), the effluent concentration of AgNPs and TiO\(_2\)NPs were 0.83 mg L\(^{-1}\) and 0.058 mg L\(^{-1}\), respectively, while in the DI water, their concentrations were 3.3 mg L\(^{-1}\) and 0.24 mg L\(^{-1}\), respectively.

Fig. 3 shows the change in the average sizes of NPs (measured as HDD) exposed to the synthetic wastewater as a function of contact time (up to 24 h). As shown Fig. 3, the HDD of all three NPs significantly increased. For instance, the HDD values of the AgNPs and TiO\(_2\)NPs increased from ca. 62 nm and 202 nm to 110 nm and 250 nm, respectively, and the size of the SiO\(_2\)NPs increased from 171 nm to 1200 nm after 9 h. Overall, this growth in average NP size appeared to have increased removal of the NPs except SiO\(_2\)-NPs. This increase in NP size is likely due the electrostatic double layer compression of NPs, which results from the ionic strength of synthetic wastewater and the consequential formation of aggregates (Badawy et al., 2010; Bae et al., 2011; Liu et al., 2011). At present, no good explanation for the reverse trend of SiO\(_2\)-NPs removal is available.

3.4. Contribution of EPS on nanoparticle removal by activated sludge

The presence of EPS is considered one of the most important features of activated sludge. Fig. 4 shows the effect of EPS presence (‘‘EPS+’’ and ‘‘EPS−’’ indicate with or without EPS, respectively) on NP removal by activated sludge under two different water conditions (DI water in Fig. 4a and synthetic wastewater in Fig. 4b). Note
that EPS in the activated sludge contained ca. 13 mg L\(^{-1}\) polysaccharide at a MLSS concentration of 3000 mg L\(^{-1}\) (data not shown). As shown in Fig. 4, the removal of the three NPs in the absence of EPS were higher than that in the presence of EPS under both water conditions, indicating that EPS plays an important role in the removal of the NPs by activated sludge. The increased removal of NPs in the presence of EPS can be explained by the enhancement of NPs with EPS and subsequent removal by sedimentation.

Activated sludge exposed to the NPs was imaged by TEM analysis. Fig. 5 shows TEM images of the interaction between activated sludge and NPs in the presence (a–d) and absence of EPS (e–h). These TEM results clearly show the different mechanisms for NP removal by EPS. For example, in the case of AgNPs, the AgNPs appeared disperse within the EPS matrix (Fig. 5b), while in the absence of EPS, the NPs were adsorbed to the surface of the microorganisms (Fig. 5f). On the other hand, TiO\(_2\)NPs and SiO\(_2\)NPs appeared as aggregates within the EPS matrix (Fig. 5b) or extracellular space (Fig. 5c and d), while the two oxide NPs existed as aggregates in the extracellular space (Fig. 5g and h) to a less extent than that in the presence of EPS. These observations support the hypothesis that in the presence of EPS, NP entrapment in the EPS matrix was one of the major mechanisms of NP removal, while, regardless of the presence of EPS, the removal of AgNPs are only affected by NP adsorption onto microorganisms which can be explained by the electrical double layer compression of both NPs and microorganisms.

However, this observation is in contrast with a previous study, which reported little contribution of EPS on AgNPs and TiO\(_2\)NPs removal by activated sludge (Kiser et al., 2010). These differences were observed at a low concentration of activated sludge including EPS (400 mg L\(^{-1}\) total suspended solids, TSS), which is far from the actual on-site conditions present during activated sludge processes (1000–5000 mg L\(^{-1}\) TSS).

4. Conclusions

In this study, the removal of engineered NPs (AgNPs, TiO\(_2\)NPs, and SiO\(_2\)NPs) by activated sludge was investigated. All three NPs employed in this study were readily removed by the activated sludge. However, the removal efficiencies appeared to be different and were affected by the NP stability, which was related to the HDD and zeta potential. The removal of all three NPs by the activated sludge occurred in a time-dependent manner, implying the importance of the HRT during the activated sludge process on the NP removal efficiency. In addition, the presence of both a synthetic wastewater component and EPS significantly enhanced the NP removal efficiency. This results indicate that the increase in the HDD of the NPs by electrical double layer compression or the entrapment of NPs by EPS may contribute to NP removal by activated sludge.

Even though the removal of NPs was greatly enhanced by the activated sludge, the possibility that NPs were present in the effluent cannot be excluded. Hence, the removal efficiency should be enhanced by controlling the operating conditions of the activated sludge process, the conditions of the activated sludge, and employing additional processes for NP removal during the treatment process.

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