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Fate of fluorescent core-shell silica nanoparticles during simulated secondary wastewater treatment

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Abstract

Increasing use of silica nanoparticles (SiO₂ NPs) in consumer products and industrial processes leads to SiO₂ NP discharge into wastewater. Thus, there is a need to understand the fate of SiO₂ NPs during wastewater treatment. However, the detection of SiO₂ NPs in environmental systems is hindered by the elevated background levels of natural silicon. In this work, laboratory-synthesized fluorescent core-shell SiO₂ NPs were used to study the fate of these NPs during secondary wastewater treatment. Fluorescent measurements provided an easy and fast method for SiO₂ NP tracking. A laboratory-scale activated sludge system consisting of an aeration tank and a settler was fed with synthetic wastewater containing ca. 7.5 mg L⁻¹ of fluorescent SiO₂ NPs for 30 days. SiO₂ NPs were effectively removed from the wastewater (>96%) during the first 6 days, however the concentration of SiO₂ NPs in the effluent gradually increased afterwards and the NP discharge was as high as 65% of the input after 30 days of NP dosing. The poor removal of the SiO₂ NPs was related to the high colloidal stability of the NPs in the wastewater and their limited propensity to biosorption. Although some degree of NP adsorption on the biomass was observed using fluorescence microscopy, the affinity of SiO₂ NPs for the activated sludge was not enough for a sustained and effective removal of the SiO₂ NPs from the wastewater.

Graphical abstract

Keywords

Activated sludge; CMP; Engineered nanomaterials; Municipal wastewater treatment; Nanoparticle adsorption; SiO₂

1. Introduction

The increased use of engineered nanomaterials in industrial applications and consumer products has raised concerns about their health and environmental safety. Nano-sized silica (SiO_2) is among the most common nanomaterials used, with an estimated annual production worldwide exceeding 1.5 million metric tons (Holden et al., 2014). SiO_2 nanoparticles (NPs) are used in construction materials such as concrete and ceramics, in biomedical applications as drug and label carriers, or as filler materials in food packaging. SiO_2 NPs are also utilized in large quantities as abrasives in polishing slurries that are applied in chemical-mechanical planarization (CMP) processes during the manufacturing of semiconductor materials (Singh et al., 2002). CMP operations are a major source of wastewater in semiconductor manufacturing plants and it has been estimated that the SiO_2 NP concentration in CMP effluents may range from 1.3 – 8.5 g L⁻¹ (Liu et al., 2014). SiO_2 NPs have shown adverse effects in a number of *in vitro* and *in vivo* toxicity studies performed in mammal cells, mice and aquatic organisms (e.g. algae, mussels) (Canesi et al., 2010; Karlsson et al., 2014; Napierska et al., 2010; Van Hoecke et al., 2008). Consequently there is great interest to understand the fate and behavior of SiO_2 NPs from the source point to the environment.

Industrial and municipal wastewater discharges are considered primary sources of NPs to the environment (Boxall et al., 2007). However, there are not specific water regulations limiting the release of NPs to sewers or the environment. Although release of some NPs may be limited by regulations that control other water quality parameters (e.g. turbidity), it is unclear if those are enough to prevent potential risks associated with NPs. Likewise, the capacity of current wastewater treatment plants (WWTPs) to deal with NP contaminants is uncertain. Despite the high utilization of SiO_2 NPs, very little is known about the fate of these engineered NPs during municipal wastewater treatment. Only a few studies have addressed this question. For example, Jarvie et al. (2009) investigated the fate of SiO_2 NPs during simulated primary wastewater treatment, while other studies explored the interaction of SiO_2 NPs with heterotrophic biomass in short-term batch assays (Grass et al., 2014; Kiser et al., 2010; Rottman et al., 2012). These investigations provide valuable information about the interactions of SiO_2 NPs with wastewater and biosolids, however they are insufficient to predict the fate of SiO_2 NPs during biological wastewater treatment that involves long-term operation of continuous-flow bioreactor systems.

Research on the fate of SiO_2 NPs during wastewater treatment is complicated by interference of the background silicon (Si) with the measurements of SiO_2 NPs. Dissolved Si is present in natural water and wastewater at relatively high and quite variable concentrations. For example, the range of dissolved Si concentration in different natural waters measured in several countries ranged from 0.9 to 23 mg Si L⁻¹ (Georg et al., 2006; Jansen et al., 2010). These high background Si concentrations complicate the monitoring of SiO_2 NPs by means of elemental analysis. Thus, to avoid interferences with SiO_2 NP measurement we propose the use of fluorescent labeled SiO_2 NPs. Fluorescent-doped NPs are commonly used in biomedical applications for imaging purpose (Senarath-Yapa et al., 2007) since these NPs provide an easy approach to follow the path of NPs into the cell. However, few studies have used fluorescent labeled NPs to monitor their fate in environmental systems. One exception is a recent study that effectively used three types of

labeled SiO₂ NPs, including fluorescent-labeled NPs, to evaluate their transport in saturated sand (Vitorge et al., 2014).

The aim of this work was to investigate the fate of SiO₂ NPs during secondary wastewater treatment in a laboratory-scale activated sludge unit. Fluorescent core-shell SiO₂ NPs were synthesized and used in the study to facilitate the traceability of the NPs. These NPs contain a core of fluorescent dye covalently bound to SiO₂ and an outer shell of pure SiO₂, so the surface chemistry of the NPs is not modified by the organic functional groups of the dye.

2. Materials and methods

2.1. Synthesis of fluorescent core-shell SiO₂ NPs

Fluorescent core-shell SiO₂ (f-SiO₂) NPs were synthesized using a reverse microemulsion method adapted from a previously described protocol (Jin et al., 2012; Senarath-Yapa et al., 2007). First, rhodamine B isothiocyanate (Rh-B ITC) was linked to (3-aminopropyl)triethoxysilane (APTES) by mixing 15 mg Rh-B ITC and 0.15 mL APTES in 7.5 mL of n-hexanol. The mixture was stirred for 12 h at room temperature ($25 \pm 1^\circ\text{C}$). Next, 5.0 mL of the resulting Rh-B ITC/APTES conjugate was added to a microemulsion solution containing 75 mL of cyclohexane, 13 mL of n-hexanol, 17.7 mL of Triton X-100, and 5.46 mL of deionized (DI) water, and stirred for 15 min. Then, 0.9 mL of tetraethyl orthosilicate (TEOS) was added and stirred for another 30 min. Finally, 0.6 mL of NH₄OH (28–30%) was added to initiate the hydrolysis of TEOS and Rh-B ITC/APTES. The reaction mixture was stirred for 12 h at room temperature followed by the addition of 80 mL of acetone to disrupt the inverse micelles. NPs were recovered by centrifugation (10,000 g for 10 min) and washed once with DI water. Then, NPs were suspended in 5.46 mL of DI water and sonicated to improve the dispersion of the particles. This NP suspension was subjected to a capping step where a shell of pure SiO₂ was added to the surface of the NPs. For that, the NP suspension was added to the microemulsion solution previously described, except for the DI water that now was added together with the particles. After stirring for 14 h, the capped f-SiO₂ NPs were separated and washed 3 times each with ethanol and DI water, as describe before. Finally, the NPs were resuspended in 6 mL of DI water and this suspension was utilized as stock dispersion in further experiments. A total of 4 batches of f-SiO₂ NPs were used during the study.

2.2. Size and surface characterization of f-SiO₂ NPs

Transmission electron microscopy (TEM) was used to analyze the morphology and primary particle size of f-SiO₂ NPs. TEM images were acquired on a Hitachi H-8100 TEM instrument (Hitachi, Tokyo, Japan) at 200 kV. NPs were suspended in isopropanol and sonicated in an ultrasonic bath for 5 min before analysis.

The hydrodynamic particle size distribution (PSD) of f-SiO₂ NP suspensions in DI water (100 mg L⁻¹) and synthetic wastewater (7.5 mg L⁻¹) was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, Westborough, MA, US). The unit employs a 4 mW He-Ne laser with a wavelength of 633 nm, and a measurement angle of 173°.

The zeta potential (ζ -potential) of f-SiO₂ NPs dispersions was measured with the same instrument using laser Doppler velocimetry and the Smoluchowski equation to correlate particle electrophoretic mobility to ζ -potential. Additionally, the surface charge of f-SiO₂ NPs was characterized by measuring the ζ -potential in a pH range (2–10) and compared to that of pure SiO₂ NPs (10–20 nm, purity of 99.5%; Sigma-Aldrich, St. Louis, MO, US). The pH of f-SiO₂ dispersions (100 mg L⁻¹) was adjusted to the desired value using an automatic titrator (MPT-2, Malvern Instruments) connected to the Zetasizer instrument. The solutions used for pH adjustment were 0.1 M HCl, 0.1 M NaOH, and 0.01 M NaOH.

2.3. Evaluation of fluorescent dye leakage

Samples of f-SiO₂ NPs were dispersed in DI water (≈ 10 mg L⁻¹) and incubated at room temperature under continuous shaking for 15 days to evaluate potential leakage of the fluorescent dye from the core-shell structure. Samples were periodically analyzed for fluorescence intensity of the total dispersion and the fluorescence remaining after filtration of the NPs using a 25-nm membrane (Millipore, Billerica, MA, US).

2.4. Laboratory-scale secondary wastewater treatment

The fate of f-SiO₂ during secondary wastewater treatment was evaluated in a treatment unit designed according to the Organization for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals (OECD, 2001). The treatment system consisted of an aeration tank (1.1 L) and a settler (0.66 L) operated in the dark at room temperature ($25 \pm 1^\circ\text{C}$). Two air pumps provided air and mixing for the bioreactor and recirculation of the settled sludge. The aeration tank was inoculated with approximately 2.0 g volatile suspended solids (VSS) per liter of activated sludge obtained from a local sewage treatment plant (Ina Road, Tucson, AZ, US). The reactor was continuously fed with synthetic wastewater prepared according to the OECD guidelines and contained (mg L⁻¹ of tap water): peptone (160), beef extract (110), urea (30), NaHCO₃ (150), K₂HPO₄ (28), NaCl (7), CaCl₂·2H₂O (4), and Mg₂SO₄·7H₂O (2). The average chemical oxygen demand (COD) was 263 ± 61 mg L⁻¹ and the pH was 7.4 ± 0.2 . The average hydraulic retention time (HRT) of the aeration tank and the settler was 11.1 ± 1.2 and 6.8 ± 0.8 h, respectively. To maintain a concentration of VSS of approximately 2.0 g L⁻¹ in the aeration tank, 180 mL of mixed liquor were periodically withdrawn and replaced with synthetic wastewater. The average solids content in the aeration basin was 2.1 ± 0.3 g L⁻¹ VSS and 2.3 ± 0.3 g L⁻¹ TSS.

The reactor was operated for 28 d before the f-SiO₂ NPs were added. Then, an average concentration of 7.9 ± 1.7 mg L⁻¹ of f-SiO₂ NPs was supplemented to the influent. Finally, the NP addition was interrupted after 30 d of operation with f-SiO₂ NPs.

The performance of the reactor was monitored by measuring the COD concentration, the pH, and the f-SiO₂ content in the influent of the aeration tank and effluent of the settler. Additionally, the TSS and VSS in the aeration tank were periodically monitored.

2.5. Settleability of f-SiO₂ NPs in synthetic wastewater

Samples of f-SiO₂ NPs (≈ 7.5 mg L⁻¹) were incubated in sludge-free synthetic wastewater and allowed to settle for 6 h. The concentration of f-SiO₂ in the supernatant was measured

by fluorescence at the initial time and after 6 h, which is approximately the HRT maintained in the settler.

2.6. Association of f-SiO₂ NPs with the activated sludge

The association of the f-SiO₂ NPs with the biomass in the bioreactor was imaged using fluorescence microscopy. Samples of biosolids were taken from the aeration tank on day 57 and washed once with DI water. The sample was applied to a glass slide and then observed using an epifluorescence spinning disc confocal microscope (3i Intelligent Imaging Innovations, Denver, CO, US). Both, transmitted light and fluorescence images were captured for each slide. For fluorescence imaging, the laser excitation source was set to 561 nm.

2.7. Analytical methods

Si in the stock dispersions was analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES Optima 2100 DV, Perkin Elmer, Waltham, MA, US) at a wavelength of 251.611 nm and with a detection limit of 10 µg L⁻¹. Prior to metal analysis, SiO₂ samples were subjected to microwave-assisted acid digestion (MARS System, CEM Corp., Matthews, NC, US) using 1% HF (10 mL). The microwave-assisted digestion conditions were 45 min at 120°C (25 min ramp time) with a power level of 1600 W. Digested samples were then diluted in 0.1% (v/v) HF for ICP-OES analysis.

Fluorescence measurements of f-SiO₂ were performed using a fluorescence spectrometer (LS 55, Perkin Elmer) at wavelengths of 559/579 nm (excitation/emission). The fluorescence excitation and emission spectra of the f-SiO₂ NPs is shown in Fig. A1 in the Appendix.

TSS and VSS were measured according to EPA method ESS 340.2. Measurements of pH and COD (digestion method with dichromate) were conducted according to standard methods (APHA, 2005).

3. Results and discussion

3.1. Size characterization of f-SiO₂ NPs

The size of the f-SiO₂ NPs was measured by using TEM images and DLS analysis, which allowed for the determination of the primary and hydrodynamic particle size, respectively. Fig. 1 shows a representative TEM image of the f-SiO₂ NPs synthesized. The particle morphology is homogeneous and mostly spherical. The primary particle size determined by TEM was in the range of 78–88 nm for the four batches used in the study (Table 1), which confirms good reproducibility among batches. On the other hand, the average hydrodynamic size of f-SiO₂ NPs dispersed in DI water was of 254 and 237 nm (Table 1), as measured by DLS in batches II and III, respectively, which indicates a slight particle aggregation in aqueous suspensions. The hydrodynamic size of f-SiO₂ NPs was also measured in the influent of the bioreactor. The average aggregate size of f-SiO₂ NPs in synthetic wastewater, except for batch I, was slightly smaller than in DI water and it ranged between 168 – 178 nm (Table 2).

3.2. ζ -potential characterization of f-SiO₂ NPs

The surface charge of particles is an important factor influencing the fate of engineered NPs in the environment (Nowack and Bucheli, 2007), as it affects the colloidal stability and the interaction of the NPs with their surroundings (e.g. organic matter, microorganisms, clay surfaces, etc.). The surface charge of the synthesized f-SiO₂ NPs was characterized by measuring the ζ -potential over a range of pH and compared to that of pristine commercial SiO₂ NPs. In general, there was a good agreement between the ζ -potential of f-SiO₂ NPs and commercial SiO₂ NPs, and a small divergence between the data was only evident at the lowest (<3) and highest (>8) pH values (Fig. 2). These results indicate a successful capping of the fluorescent core with pure SiO₂. Thus, the behavior of f-SiO₂ NP dispersions is expected to be similar to those of pure SiO₂ NPs.

Additionally, the ζ -potential is an indicator of the colloidal stability of NP dispersions. The highly negative ζ -potential values observed over most of the studied pH range suggest a high colloidal stability of the NPs synthesized. For example, the average ζ -potential ranged between -39.2 and -47.3 mV for dispersions of f-SiO₂ NPs in DI water at $\text{pH } 7.4 \pm 0.3$ (Table 1). Likewise, the average ζ -potential in samples from the bioreactor influent ($\text{pH} = 7.3 \pm 0.2$) ranged between -15.2 and -22.4 mV (Table 2). The difference in ζ -potential values is due to the interaction of the NPs with organic and inorganic constituents in the wastewater.

3.3. Fluorescence characterization of f-SiO₂ NPs

A representative calibration curve of fluorescence intensity as a function of the NP concentration is provided in Fig. 3A. Fluorescence intensity was linear with the concentration in the entire range of concentrations used. A comparison of the NP fluorescence intensity with the background fluorescence signal shown by the synthetic wastewater (represented with a horizontal dashed line in Fig. 3A) provides a detection limit in the range of 0.5 mg L^{-1} f-SiO₂ NPs.

The fluorescence intensity of f-SiO₂ NPs in water was measured over a period of time. In addition, the fluorescence of filtered samples (<25 nm) was measured to test for potential leakage of dye from the particle. The NP fluorescence did not significantly change over a period of 15 d (Fig. 3B). Likewise, the fluorescence of filtered samples was quite constant over time. Although there was a small increase in fluorescence on day 8 of incubation, it is difficult to attribute it to dye leakage, since the fluorescence decreased close to the initial value on day 15.

3.4. Fate of f-SiO₂ during secondary wastewater treatment

Fluorescence measurements were effective for tracking f-SiO₂ NPs in the secondary wastewater treatment system. Fig. 4 shows the dynamics of f-SiO₂ NP concentration in the influent and effluent of the activated sludge unit. The bioreactor was operated in absence of NPs until day 28. Then, during the first 6 days after NP addition, no f-SiO₂ was detected in the effluent. However, a gradual increase of f-SiO₂ NP concentration in the outflow was observed after day 35, and the NP discharge was as high as 65% of the input right before the interruption of the f-SiO₂ NP dosing (day 57). Finally, the f-SiO₂ in the effluent rapidly

decreased once the bioreactor was fed with synthetic wastewater lacking NP. Over the 67 days of operation, the retention of f-SiO₂ NPs inside the bioreactor accounted for ca. 61% of the total input. However, the gradual increase of the NP concentration in the effluent with increasing operation time suggests that the capacity of the activated sludge to retain SiO₂ NPs is limited and insufficient to sustain high and constant removal of SiO₂ NPs from the wastewater.

To our knowledge, this is the first time that the fate of SiO₂ NPs in secondary wastewater treatment is evaluated in a continuous system. Other inorganic NPs have been used in similar studies with different results. Several studies have described extensive removal of CeO₂ and TiO₂ NPs (94–98%) during laboratory-scale secondary wastewater treatment (Gomez-Rivera et al., 2012; Limbach et al., 2008; Wang et al., 2012). For instance, a sustained removal of 96% of CeO₂ NPs was achieved in an activated sludge system operated for up to 60 d (Gomez-Rivera et al., 2012), and approximately 98% TiO₂ NP removal was obtained in a sequential batch reactor (SBR) dosed with NPs for 15 d (Wang et al., 2012). Additional studies were performed to address the different possible mechanisms of NP removal.

3.5. Mechanisms of f-SiO₂ NP removal during secondary wastewater treatment

In conventional secondary wastewater treatment, NPs can potentially be removed via either sedimentation (discrete or flocculant), or partitioning onto the biomass (Westerhoff et al., 2013). Settleability tests performed in synthetic wastewater free of microbial biomass indicated that the amount of f-SiO₂ NPs removed by settling after 6 h was less than 2% (Fig. A2 in the Appendix). Thus, removal of f-SiO₂ NPs in the secondary clarifier, which was operated at a HRT of 6.8 h, by means of gravitational settling is expected to be insignificant. These results are in agreement with the negligible removal reported for SiO₂ NPs (56 nm) spiked into real sewage during simulated primary settling (3 h) (Jarvie et al., 2009). In general, SiO₂ NPs display very high colloidal stability when dispersed in different matrices such as wastewater, DI water or tap water, which prevents significant NP aggregation and settling (Otero-González et al., 2014; Zhang et al., 2008). The high colloidal stability of SiO₂ NPs at the circumneutral pH values typical of municipal wastewater is due to the extremely low point of zero charge (PZC) of SiO₂ (below 3.5) (Otero-González et al., 2014). In contrast with SiO₂, engineered NPs with PZC values which are close to the pH of municipal wastewater such as CeO₂ (PZC ≈ 7.0) (Otero-González et al., 2014) and TiO₂ (PZC ≈ 5.3) (Wang et al., 2012) and have been shown to be highly removed during aerobic biological wastewater treatment (Gomez-Rivera et al., 2012; Wang et al., 2012). The average hydrodynamic sizes determined in the later studies for CeO₂ (primary size = 50 nm) and TiO₂ NPs (primary size = 20 nm) after the NPs were suspended in wastewater was as high as 2500 and 1700 nm, respectively (Gomez-Rivera et al., 2012; Wang et al., 2012). Therefore, sedimentation of the micron-sized aggregates would be expected to play an important role in the removal of both nanomaterials. Indeed, Wang et al. (2012) observed a high removal of TiO₂ NPs (65%) in a SBR operated in the absence of microbial biomass. The removal of TiO₂ NPs further increased to 98% in the presence of sludge (Wang et al., 2012).

The high colloidal stability of SiO₂ NPs suggests that the removal of this nanomaterial during secondary wastewater treatment must be dominated by interactions with the activated sludge. Indeed, microscopic examination of a sample of mixed liquor obtained from the aeration tank on day 57 confirmed the presence of f-SiO₂ NPs in the microbial flocs (Fig. 5). The overlay of the fluorescence and transmitted light microscopy images clearly shows how most of the fluorescence intensity (red color) is displayed in areas where biomass is present (Fig. 5C). Although a fraction of the f-SiO₂ NPs was removed by biosorption, the rapid deterioration in the ability of the bioreactor to remove SiO₂ NPs suggests that the capacity of the biomass to adsorb the NPs was low and that the growth of new sludge was insufficient to maintain high levels of NP removal. Indeed, the low affinity of aerobic sludge for SiO₂ NPs has been confirmed in two previous batch isotherm studies which observed that, at concentrations below 20 mg SiO₂ L⁻¹, the distribution of SiO₂ NPs between the liquid phase (i.e., either bicarbonate or phosphate buffer) and the biomass could be fitted to linear Freundlich models with very low K_d values, 0.124 and 0.613 L g⁻¹ VSS, respectively (Kiser et al., 2010; Rottman et al., 2012). The former K_d value was estimated from the data reported by Kiser et al. (2010), 0.105 L g⁻¹ TSS, assuming that the VSS content of the sludge was 85% of the total dry solids.

The low affinity of SiO₂ NPs for the activated sludge can be, in part, explained by electrostatic interactions. The extremely low PZC of SiO₂ NPs (below 3.5) indicates a relatively high negative surface charge at the operational pH (7.4 ± 0.2). Likewise, the surface of activated sludge is mainly negatively charged at circumneutral pH values (Steiner et al., 1976). Thus, electrostatic repulsion among SiO₂ NPs and activated sludge will dominate. Nevertheless, activated sludge flocs contain a wide array of components (bacteria, inorganic particles, and extracellular polymeric substances), which confer the sludge with heterogeneous surface properties and allows for the presence of hydrophobic regions (Liao et al., 2001). These regions may serve as zones of low SiO₂ NP repulsion, where NPs may adhere to the sludge surface. Moreover, the variability in the chemical composition of real wastewater may affect the surface charge of SiO₂ NPs and microbial biomass, and modify the electrostatic interactions between them. For example, higher concentrations of divalent cations such as Mg²⁺ and Ca²⁺ in wastewater could favor attractive forces and biosorption of NPs (Zhang et al., 2008; Zita and Hermansson, 1994). Finally, other mechanisms such as entrapment into flocs may contribute to the partitioning of SiO₂ NPs to the biomass (Westerhoff et al., 2013).

In contrast with the low affinity of SiO₂ NPs for biosorption by aerobic biomass observed in the current investigation and other literature reports (Kiser et al., 2010; Rottman et al., 2012), a recent batch study reported over 97% removal of SiO₂ NPs encapsulating a DNA sequence (0.01–10 mg L⁻¹) which was attributed to adsorption onto the activated sludge (Grass et al., 2014). Direct comparison with results of our study is complicated by the fact that the sorption experiments performed by Grass et al. (2014) were conducted in short-term assays (2 h) and used real municipal wastewater, as opposed to the synthetic wastewater, which may account for differences in the propensity of NP to biosorption, since variations in the organic and ionic content in wastewater affect their surface charge. Differences in the physicochemical properties of the nanomaterials used may also account for the discrepancy. The SiO₂ particles used by Grass et al. (2014) included a large fraction (≈ 78%) with a

diameter above 100 nm in contrast to average primary particle sizes below 100 nm in the other studies. Smaller NPs may have higher colloidal stability and, therefore, persist longer in suspension.

3.6. Effect of f-SiO₂ NPs in COD removal

Another concern about the release of NPs to WWTPs is a potential negative impact on the microorganisms responsible for wastewater treatment. Fig. 6 shows the COD removal attained in the activated sludge unit during the operational time. The average COD removal attained by the bioreactor before the addition of f-SiO₂ NPs ($93 \pm 5\%$) remained almost constant after f-SiO₂ NPs were dosed ($94 \pm 7\%$). These results are in agreement with a study that did not detect any adverse effect on cell viability and membrane integrity of activated sludge after acute (1 d) or chronic (70 d) exposure to SiO₂ NPs up to concentrations of 50 mg L⁻¹ (Zheng et al., 2012). SiO₂ NPs have also shown low toxicity to model bacteria. For example, a concentration as high as 1 g L⁻¹ of SiO₂ NPs only caused 7% and 19% cell growth inhibition of *Bacillus subtilis* and *Escherichia coli*, respectively, after a 6-h exposure period (Adams et al., 2006). The results suggest that moderate concentrations of SiO₂ NPs are not expected to cause COD removal impairment in activated sludge systems.

3.7. Implications

The results of this study indicate that a simulated activated sludge treatment system operated under conditions representative of those applied in full-scale WWTPs was not effective for the sustained removal of SiO₂ NPs from wastewater. Thus, SiO₂ NPs discharged to municipal sewer systems will likely be released to the environment along with the treated effluent from conventional municipal WWTPs. This may be of special concern if the receiving water body is used as a source of drinking water. Although the health hazards of SiO₂ NPs are still poorly understood, the results obtained in a growing number of *in vitro* and *in vivo* studies have shown that exposure to some SiO₂ NPs can lead to adverse effects in aquatic organisms and mammals (Canesi et al., 2010; Karlsson et al., 2014; Napierska et al., 2010; Van Hoecke et al., 2008). Moreover, some authors have observed an increase in the toxicity of SiO₂ NPs with decreasing particle size which was attributed to the reduced size and/or the larger specific surface area of smaller particles (Napierska et al., 2010; Van Hoecke et al., 2008). Thus, the poor effectiveness of conventional WWTPs to remove smaller SiO₂ NPs (typically < 100 nm) like the ones used in this study may implicate a health and ecotoxicological hazard.

Although continuous-flow studies investigating the fate of SiO₂ NPs in real municipal wastewater during activated sludge treatment are lacking, the results of two previous studies suggest that, similarly to the results obtained with synthetic wastewater, SiO₂ NPs will likely be poorly removed. Jarvie et al. (2009) observed very low removal of SiO₂ NPs during simulated primary settling of real municipal wastewater. Likewise, SiO₂ NPs in primarily-treated municipal wastewater were found to be very stable in batch assays reported in our previous study (Otero-González et al., 2014).

Different alternatives may be considered for the removal of SiO₂ NPs from wastewater: 1) NP removal at the point of use (e.g. semiconductor manufacturing facilities); 2) re-design of

conventional municipal WWTPs for NP removal (e.g. including specific tertiary treatments); and 3) NP removal at drinking water treatment facilities. The first alternative is of particular interest to industries such as the semiconductor manufacturing due to the possibility of recycling treated water into the production process. Moreover, different treatment methods such as electrocoagulation and ultrafiltration are being proposed for the removal of SiO₂ NPs from highly concentrated wastewater with satisfactory removal efficiencies (Den and Huang, 2005; Springer et al., 2013). Regarding the second and third alternatives, more research is needed to elucidate the capacity of current tertiary treatment methods and existing drinking water facilities for the effective removal of SiO₂ NPs.

4. Conclusions

Fluorescent core-shell SiO₂ NPs were successfully synthesized and used for tracking the fate of SiO₂ NPs during simulated secondary wastewater treatment. The results indicate that conventional activated sludge treatment may not be sufficient to provide sustained and effective removal of moderate concentrations of SiO₂ NPs from wastewater. The high colloidal stability and negatively charged surface area of SiO₂ NPs impeded their removal either by sedimentation or partition to the biomass. Nevertheless, some adsorption of SiO₂ NPs onto the biomass was observed. Finally, SiO₂ NPs did not cause any impairment of the COD removal capacity of the system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2015.03.021>.

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Highlights

- Fluorescent SiO₂ NPs were used to track their fate in activated sludge treatment.
- A significant amount of SiO₂ NPs escaped with the treated effluent.
- Some NP adsorption on the biomass was observed using fluorescence microscopy.
- Exposure to SiO₂ NPs did not affect the COD removal.

Fig. 1. Transmission electron microscopy image of the fluorescent core-shell SiO₂ NPs synthesized and used in this study.

Fig. 2. ζ -potential of f-SiO₂ (●) and commercial SiO₂ (□) NP dispersions (100 mg L⁻¹) in deionized water as a function of pH.

Fig. 3.

(A) Fluorescence calibration curve for the f-SiO₂ NPs suspended in synthetic wastewater. The best fit regression line was $Fluorescence = 21.5 \cdot Concentration + 20.4$ ($R^2 = 0.9998$). The dashed horizontal line indicates the background fluorescence measured for synthetic wastewater. (B) Fluorescence intensity over time of f-SiO₂ NP dispersions in deionized water incubated at room temperature and under continuous shaking. The chart shows the total fluorescence of the sample (●) and the remaining fluorescence after membrane filtration by 25 nm (□). The dashed horizontal line indicates the background fluorescence measured for DI water. Error bars represent the standard deviation between duplicate samples.

Fig. 4. Concentration of f-SiO₂ in the influent (●) and effluent (□) of the laboratory-scale activated sludge unit. The dashed vertical lines delimitate the period when f-SiO₂ NPs were added to the influent. Error bars represent the standard deviation between duplicate samples.

Fig. 5.
(A) Transmitted light microscopy image of a sample of mixed liquor obtained from the aeration tank on day 57; (B) Fluorescence microscopy image of the same sample; (C) overlay of the two previous images, A and B.

Fig. 6. Total COD removal in the activated sludge unit. The dashed vertical lines delimitate the period when f-SiO₂ NPs were added to the influent.

Table 1

Summary of the particle size and ζ -potential characterization of the various batches of f-SiO₂ NPs used in the study. The table contains the primary particle size calculated following TEM imaging, and the hydrodynamic particle size, ζ -potential, and point of zero charge of NP dispersions ($\approx 100 \text{ mg L}^{-1}$) in DI water ($\text{pH} = 7.3 \pm 0.2$).

Batch	Primary particle size (nm)	Hydrodynamic Particle size (nm)	ζ -Potential (mV)	PZC (pH)
I	78 ± 6	ND ^a	-47.3 ± 0.1	<3.16
II	88 ± 7	254 ± 5	-42.1 ± 2.0	3.19
III	84 ± 7	237 ± 9	-39.2 ± 3.3	3.27
IV	81 ± 6	ND	-46.7 ± 0.6	3.23

^aND: Not determined.

Table 2

Summary of the hydrodynamic particle size and ζ -potential of the different batches of f-SiO₂ NPs dispersed in synthetic wastewater ($\approx 7.5 \text{ mg L}^{-1}$, pH = 7.3 ± 0.2).

Batch	Hydrodynamic Particle size (nm)	ζ -Potential (mV)	Polydispersity Index
I	400 ± 35	-15.4 ± 1.1	0.406 ± 0.037
II	178 ± 2	-20.8 ± 1.1	0.172 ± 0.024
III	169 ± 4	-22.4 ± 1.3	0.188 ± 0.005
IV	168 ± 2	-21.2 ± 2.1	0.181 ± 0.007