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Effect of silver nanoparticles on system performance and microbial community dynamics in a sequencing batch reactor

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ABSTRACT

The effect of silver nanoparticles on the system performance and the bacterial community dynamics in a sequencing batch reactor was investigated, along with the removal and the fate of silver nanoparticles within the system. Results showed that silver nanoparticles did not significantly affect organic matters removal, nitrification and denitrification. However, adverse effects were observed on sludge settleabilities, which were related to the over-production of extracellular polymeric substances and a sequential change in activated sludge structure and morphology. Polymerase chain reaction–Denaturing gradient gel electrophoresis revealed that silver nanoparticles caused significant shifts in bacterial community structures. Different species were affected and eliminated at each dosage of silver nanoparticles. Silver nanoparticles were effectively removed in the bioreactor, with the main removal pathway being deposited in the activated sludge.

1. Introduction

Nanomaterials exhibit very different physical and chemical properties from their bulk counterparts due to their small size (with at least one dimension between 1 and 100 nm) and large specific surface area (>60 m^2/cm^3) (Kreyling et al., 2010). Synthetically manufactured nanoparticles (NPs) exploits these advantages and are becoming increasingly common in consumer products (Bauer et al., 2008). For example, silver nanoparticles (Ag NPs), due to its excellent antimicrobial activities, are widely incorporated into many consumer products, such as clothing, paints, bandages, and food containers (Hou et al., 2012). However, these wide ranging applications of Ag NPs also increase their risk of potential release into the environment. Material-flow modelling studies suggested that a major flow of Ag NPs is from the production, manufacturing, and consumption to wastewater, due to Ag NPs applied to textiles and their release during washing (Sun et al., 2014). Benn and Westerhoff (2008) also showed that the Ag leached from the washing of Ag NPs coated fabrics can reach as high as 1.3 mg/L. Currently, knowledge on the concentrations of NPs in sewage wastewater is lacking due to the paucity of specific methods to distinguish and quantify it from naturally occurring nanoscale as well as its bulk materials (Tan et al., 2015). The expected Ag concentrations in municipal wastewater are in the μg/L range due to the mixing of different wastewater streams (Gottschalk et al., 2009). Nevertheless, if wastewater treatment plants receive high Ag-containing industrial effluents, Ag concentration in the influent can reach as high as mg/L level (Zhang et al., 2014). Additionally, with rapid growth in the production of Ag NPs, their high occurrence in the municipal wastewater is inevitable in the near future (Sun et al., 2014).

Municipal wastewater treatment is an essential part of any modern society in eliminating contaminants and protecting the water environment and human health. In view of the adverse effects of Ag NPs on bacteria (Choi et al., 2008) and biofilm (Sheng and Liu, 2011), there is a pressing need to investigate the potential effects of these NPs on the biological wastewater treatment processes. Additionally, as the last barrier before the treated effluent is discharged into water bodies, wastewater treatment plants also play a crucial role in controlling the entrance of these emerging pollutants into the environment. Thus, it is important to understand the fate of NPs in the treatment process and to examine their removal from wastewater.

In this work, the long-term effect of 1.0 and 5.0 mg/L Ag NPs on biological wastewater treatment was examined in a sequencing batch reactor (SBR) vis-a-vis pollutant removal efficiencies,

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activated sludge properties and bacterial community dynamics. The removal and distribution behaviour of Ag NPs in the SBR system were also investigated to elucidate the potential fate of Ag NPs during biological wastewater treatment.

2. Materials and methods

2.1. Sequencing batch reactor (SBR)

An SBR with an operating volume of 2.2 L and treating 1.54 L of wastewater per cycle was set up. Each cycle consisted of 15 min fill, 120 min anaerobic react, 120 min aerobic (I) react, 90 min anoxic react, 60 min aerobic (II) react, 45 min settle, and 30 min decant. The influent fill stage was split into 11 min of influent fill at the beginning, and 4 min of influent fill after the aerobic (I) react stage to provide addition organic material for effective denitrification. In addition, 80 ml/day of mixed liquor was withdrawn from the reactor at the end of the anoxic stage in the sludge wastage process. The SBR was operated with an initial sludge retention time (SRT) and hydraulic retention time (HRT) of 20 days and 11.7 h respectively, and was run for 65 days to achieve quasi steady state. From Day 66 to Day 93, 1.0 mg/L of Ag NPs were added to the synthetic wastewater to examine the potential effects of Ag NPs on wastewater treatment. The influent Ag NPs concentration was further increased to 5.0 mg/L from Day 94 to investigate the response of the wastewater treatment process.

2.2. Wastewater and silver nanoparticles

The influent synthetic wastewater was prepared daily. Sodium acetate, NH_4Cl and KH_2PO_4 were added to give influent chemical oxygen demand (COD), nitrogen, and phosphorus concentrations of 400 mg/L, 40 mg/L, and 8 mg/L respectively. The synthetic wastewater also contained 19.3 mg/L $CaCl_2 \cdot 2H_2O$, 71.0 mg/L $MgSO_4 \cdot 7H_2O$, 17.4 mg/L $FeSO_4 \cdot 7H_2O$, 0.07 mg/L $CuCl_2 \cdot 2H_2O$, 0.13 mg/L $MnCl_2 \cdot 4H_2O$, 0.13 mg/L $ZnSO_4 \cdot 7H_2O$, 0.03 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.025 mg/L H_3BO_3 and 0.033 mg/L KI.

A stock suspension of Ag NPs (Sigma Aldrich, Missouri, USA) was prepared by suspending 0.5 g Ag NPs per L of 0.25 mM sodium citrate solution. Particle size and zeta potential were analyzed using a Malvern Zetasizer Nano ZS (Malvern Instruments, USA). Ag NPs suspension was first diluted to 100 mg/L using Milli-Q water, followed by sonication for 1 h using Elmasonic S30H (Elma GmbH & Co, Germany) (Tan et al., 2015). The particle size of the Ag NPs suspension was determined to be 118.5 ± 1.2 nm.

2.3. Analytical methods

The concentration of wastewater pollutants and the characteristics of the activated sludge were analyzed in accordance to Standard Methods (APHA 1999). The concentration of total nitrogen in the wastewater was calculated as the sum of the concentration of ammonia–nitrogen, nitrite–nitrogen and nitrate–nitrogen in the wastewater. Wastewater analysis and sludge characterization were done at least in duplicates, with the exception of the settled sludge volume in the calculation of sludge volume index (SVI).

The total Ag concentration in the influent and effluent wastewater was determined using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent Technologies 7500 series, USA). Acid digestion of the sample wastewater was conducted using a protocol similar to 3030E of the Standard Methods (APHA, 1999). 5 ml of the sample was acidified with 1 ml of trace metal grade nitric acid and refluxed at 105 °C for 2 h. The resultant solution was filtered through a 0.45 μm filter membrane before analysis.

In addition to the wastewater, the concentration of Ag in the activated sludge was also analyzed after acid digestion (USEPA, 1995). 10 ml of mixed liquor was first centrifuged at 5000 rpm for 5 min and the supernatant discarded before the sample was washed with Milli-Q water. 5 ml of nitric acid was added to the residue and refluxed at 105 °C for 2 h, followed by filtration through a 0.45 μm membrane filter. The resultant solution was diluted to a final volume of 10 ml using Milli-Q water.

To prepare the activated sludge for SEM analysis, sludge sample from the SBR was first washed with phosphate buffer solution (PBS) before it was soaked overnight in 2.5 wt% glutaraldehyde. The treated sample was washed again with PBS and dehydrated with an ethanol gradient (25%, 50%, 75%, 90% and 99%). The samples were resuspended in 99% ethanol before several droplets of the suspension were added to the SEM holder and dried in the desiccators (Qiu and Ting, 2013). The dried samples were platinum coated before SEM (Jeol JSM-5600LV, Japan) analysis. An EDX elemental analysis of the sludge samples was performed using an attached silicon drift detector (Oxford Instruments x-act, UK).

2.4. Soluble microbial products (SMP) and extracellular polymeric substances (EPS)

SMP and EPS produced by the bacteria were analyzed as protein and polysaccharide concentrations. SMP were obtained by centrifuging the mixed liquor at 12,000 rpm for 10 min and filtering the supernatant through a 0.45 μm membrane filter (Qiu and Ting, 2014). Milli-Q water was then added to the sludge residue before the sample was heated in a water bath at 80 °C for 30 min. The resultant solution was then centrifuged again at 12,000 rpm for 10 min and the supernatant filtered through a 0.45 μm membrane filter to obtain the EPS.

Protein concentration was analyzed using a modified Lowry procedure (Lowry et al., 1951) with Bovine Serum Albumin as standard. Polysaccharide content was analyzed using the phenol–sulphuric acid method (Woods et al., 2011) with glucose as the standard.

2.5. Sludge sampling, DNA extraction, polymerase chain reactor and denaturing gradient gel electrophoresis

10 ml mixed liquor were centrifuged (1000 g for 5 min); the supernatant was decanted and the pellet was resuspended in 10 ml sterile Tris–EDTA buffer (10.0 mM Tris–HCl, 1.0 mM EDTA, pH 8.0). All samples were immediately frozen after resuspension, and were stored at −80 °C until DNA extraction. DNA was extracted from the samples with a QIAamp DNA Mini Kit (Qiagen, Valencia, CA). To minimize variations in DNA extraction, templates used for polymerase chain reactor (PCR) amplification were prepared by mixing the DNA that was extracted in triplicate for each sample.

In order to increase the yield of PCR products and to facilitate the denaturing gradient gel electrophoresis (DGGE) analyses, a nested PCR technique was applied (Qiu and Ting, 2013). For the total bacterial community, the 16S rRNA genes were amplified from the DNA extracts using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′), following a temperature cycling conditions: Pre-incubation at 95 °C for 2 min, followed by 25 cycles of 95 °C for 1 min, 62 °C for 1.5 min, and 72 °C for 1 min; and a final elongation at 72 °C for 10 min. A nested PCR was then performed on the PCR products obtained from previously described primers with a second primer pair 357F-Clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-CCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′), following a cycling program: Pre-incubation at 95 °C for 2 min, followed by 30 cycles of 95 °C for 1 min, 60 °C for 45 s, and