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Effect of Zinc oxide nanoparticles on biological wastewater treatment in a sequencing batch reactor

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ABSTRACT

The rapidly increasing use of engineered nanoparticles (NPs) in consumer products inevitably leads to their greater presence in wastewater treatment plants. In this study, the effects of zinc oxide nanoparticles (ZnO-NPs) on system performance and bacterial community dynamics of biological wastewater treatment in a lab-scale sequencing batch reactor were evaluated, along with their fate within the system. It was found that ZnO-NPs caused poor settleability of the activated sludge and a significant decrease in the removal of nitrogen and phosphorus over time. Denaturing gradient gel electrophoresis analysis revealed that the bacterial community in the activated sludge became less diverse after exposure to ZnO-NPs. In addition, the production of extracellular polymeric substances (EPS) increased, with the EPS forming a tight matrix to protect the cells from the NPs. The NPs were removed very effectively from wastewater, mainly via sorption to the sludge.

1. Introduction

Municipal wastewater treatment, typically consisting of primary treatment using physical and chemical means, and secondary treatment using activated sludge, is indispensable in any modern city. A reduction in the treatment efficiencies in any of these treatment processes would have adverse effects on the environment and the quality of life.

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), nanoparticles (NPs) exhibit optical, electrical, and chemical characteristics different from either their bulk or dissolved forms (Heithmar and Pergantis, 2010). Synthetically manufactured NPs, also known as engineered NPs, exploit these properties and are becoming increasingly common in consumer products (Bauer et al., 2008). In particular, zinc oxide nanoparticles (ZnO-NPs) are widely used in semiconductors, sunscreens, pigments and food additives (Wu et al., 2010). The possible substitution of nanoparticles for toxics materials in manufacturing holds great promise, but also present many risks (Ellenbecker and Tsai, 2011); it has been well documented that these NPs can be hazardous to human and ecological health (Reijnders, 2006). Earlier studies have found that NPs can cause oxidative damage to bacterial and mammalian cells through the production of reactive oxidation species (Setyawatia et al., 2013). DNA damage caused by NPs may also result in carcinogenesis in cells (Ng et al., 2011). Indeed, there is urgent need for a major increase in nanomaterial risk research, as well as the development of adequate regulatory policies on nanomaterial risk management (Walsh et al., 2008).

As NPs enter wastewater streams and end up at the treatment plants, they inhibit some bacterial species in the activated sludge and result in a reduction in the efficiency in biological wastewater treatment (Wang et al., 2012). Zheng et al. (2011) showed that long-term exposure of NPs not only significantly reduced the population of ammonia-oxidizing bacteria but also inhibited the activities of ammonia monooxygenase and nitrite oxidoreductase. NPs reportedly exert different effect on different bacteria species. For example, slow growing bacteria and those that produce large amounts of extracellular polymeric substances (EPS) were found to be more resistant to NPs (Sheng and Liu, 2011). Liang et al. (2010) showed that while NPs exposure did not affect the growth of heterotrophs, the nitrifying bacteria population was significantly reduced.

Previous research has concluded that significant amounts of ZnO-NPs pass through primary treatment to enter the secondary biological treatment process (Hou et al., 2013). ZnO-NPs were also observed to exhibit significant toxicity to the activated sludge at lower concentrations compared to other NPs (Mu et al., 2011). Unlike many other common NPs, the slight solubility of ZnO-NPs

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may have contributed to its higher toxicity (Liu et al., 2011). However, not much research has been conducted on their effect on biological wastewater treatment in the long term. As wastewater treatment plants are the last barrier before the water is discharged into the environment, it is important to understand the fate of NPs in the treatment process and examine their removal from wastewater. Studies have found that biomass may remove NPs from wastewater by trapping them in the EPS or biofilm matrixes (Kiser et al., 2010). However, there is a paucity of data on the removal of ZnO-NPs during the biological wastewater treatment process, due to the dearth of specific methods for quantification of ZnO-NPs in wastewater samples and the lack of information regarding their transformation during the wastewater treatment process (Lombi et al., 2012).

In this work, long-term effects of ZnO-NPs on the biological wastewater treatment (over 62 days) were studied in a sequencing batch reactor (SBR) vis-a-vis the pollutant removal efficiencies, activated sludge properties and bacterial community dynamics. The removal and distribution behaviour of ZnO-NPs in the SBR system were also investigated to illustrate the potential fate of ZnO-NPs during biological wastewater treatment.

2. Material 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 used in this study. 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 of organic material for effective denitrification. In addition, 26.7 ml of mixed liquor was withdrawn from the reactor at the end of the anoxic stage for each cycle in the sludge wastage process (a total of 80 ml of mixed liquor withdrawn per day). The SBR was operated with an initial sludge retention time (SRT) and hydraulic retention time (HRT) of 16 days and 11.7 h respectively, and was run for 56 days to achieve steady state. From Day 57 onwards, ZnO-NPs were added to the synthetic wastewater to simulate an environmentally relevant concentration of 1 mg/L.

2.2. Wastewater and ZnO-NPs

Influent synthetic wastewater was prepared daily using tap water supplied by the Public Utilities Board, Singapore. Sodium acetate (CH_3COONa), ammonium chloride (NH_4Cl) and potassium dihydrogen phosphate (KH_2PO_4) were added to give an influent chemical oxygen demand (COD), nitrogen, and phosphorus concentrations of 650 mg/L, 40 mg/L, and 8 mg/L respectively. The synthetic wastewater also contained 19.3 mg/L calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 71.0 mg/L magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$), 17.4 mg/L iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), 0.07 mg/L copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), 0.13 mg/L manganese (II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$), 0.13 mg/L zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$), 0.03 mg/L sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$), 0.025 mg/L boric acid (H_3BO_3) and 0.033 mg/L potassium iodide (KI).

Commercially-produced ZnO-NPs as a suspension of 50 wt% ZnO in water were purchased from Sigma Aldrich, USA. Analysis of particle size and zeta potential were performed using a Malvern Zetasizer Nano ZS (Malvern Instruments, USA). ZnO-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). The particle size was determined to be 66.25 ± 36.61 nm for the freshly prepared suspension and 67.32 ± 33.68 nm for the suspension that have been left to stand for 24 h, with corresponding zeta potentials of −47.2 mV and −45.6 mV respectively.

2.3. Analyses of wastewater quality and sludge properties

The concentration of wastewater pollutants and the characteristics of the activated sludge were analysed in accordance to Standard Methods (APHA, 1999). The concentration of total nitrogen in the wastewater was calculated as the sum of ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen concentrations 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 standard deviations of the measured values were calculated and represented by the error bars in the figures. An analysis of variance (ANOVA) was also performed using SPSS 13.0 for Windows (SPSS Inc, USA). A *p-value* of less than 0.05 is taken to be statistically significant.

As ZnO-NPs are slightly soluble, both Zn^{2+} and total Zn concentrations in the influent and effluent wastewater were analysed. Analysis was conducted using an Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) (Agilent Technologies 7500 series, USA). Acid digestion of the sample wastewater was conducted in a process 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. The released Zn^{2+} due to the dissolution of ZnO NPs was determined according to the literature (Zheng et al., 2011).

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

The amount of soluble microbial products (SMP) and EPS generated by the bacteria were analysed 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 (Antonelli et al., 2011). 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 analysed using a modified Lowry procedure (Lowry et al., 1951), with Bovine Serum Albumin as standard. Polysaccharide content was analysed using the phenol-sulphuric acid method, with glucose as standard (Qiu and Ting, 2014).

To prepare the activated sludge for SEM analysis, sludge sample from the SBR was first washed with phosphate buffer solution (PBS) before being soaked overnight in 2.5 wt% glutaraldehyde. The treated sample was then washed again with PBS and dehydrated with an ethanol gradient (25%, 50%, 75%, 90% and 99%). The samples were then resuspended in 99% ethanol before several droplets of the suspension were added to the SEM specimen mount holder and dried in the desiccator. The dried samples were coated with platinum 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).