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Effect of CuO nanoparticles on the production and composition of extracellular polymeric substances and physicochemical stability of activated sludge flocs

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HIGHLIGHTS

- Effect of CuO-NPs on EPS and physicochemical stability of sludge flocs was studied.
- The production of LB-EPS (polysaccharides) was enhanced to resist the nanotoxicity.
- C—O—C and carboxyl groups in the EPS changed in the presence of nano-CuO.
- Exposure to 50 mg/L CuO NPs caused a decrease in flocculation and dewaterability.

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ABSTRACT

The effects of CuO nanoparticles (NPs) on the production and composition of extracellular polymeric substances (EPS) and the physicochemical stability of activated sludge were investigated. The results showed enhanced production of loosely bound extracellular polymeric substances (LB-EPS), protecting against nanotoxicity. Specifically, polysaccharide production increased by 89.7% compared to control upon exposure to CuO NPs (50 mg/L). Fourier transform-infrared spectroscopy analysis revealed changes in the polysaccharide C—O—C group and the carboxyl group of proteins in the EPS in the presence of CuO NPs. The sludge flocs were unstable after exposure to CuO NPs (50 mg/L) because of excess LB-EPS. This also corresponded with decreased cell viability of the sludge flocs, as determined by the production of reactive oxygen species and the release of lactate dehydrogenase. These results are key to assessing the adverse effects of the CuO NPs on activated sludge in wastewater treatment plants.

1. Introduction

With the rapid development and application of nanotechnology, a large number of nanomaterials are used in consumer and industrial products such as semiconductors, cosmetics, textiles, and pigments (Gottschalk et al., 2009). Some reports have suggested that the wide use of nanoparticles (NPs) has inevitably caused their release into the environment, such as into wastewater treatment plants (WWTPs), natural water bodies, and the soil (Nowack and Bucheli, 2007). At the same time, there are increasing concerns over the risks posed by NPs to the health of humans and the ecosystem (Sharifi et al., 2012). Therefore, it is imperative to understand the environmental impact of NPs.

WWTPs are important for preventing NPs from entering the natural environment (Nowack and Bucheli, 2007). While a significant amount of NPs can be removed from the wastewater treatment systems through biosorption using activated sludge (Kiser et al., 2010), the adsorbed NPs may induce adverse effects on microbial growth (Zhang et al., 2014). Their toxicity to some microorganisms within the biological systems of the WWTPs (Brar et al., 2010) is of particular concern. Recent studies have shown that

Abbreviations: CLSM, confocal laser scanning microscopy; COD, chemical oxygen demand; CST, capillary suction time; DCF, dichlorofluorescein; EPS, extracellular polymeric substance; ESS, effluent suspended solid; FT-IR, Fourier transform-infrared spectroscopy; LB-EPS, loosely bound EPS; LDH, lactate dehydrogenase; MLVSS, mixed liquor volatile suspended solid; NP, nanoparticle; PRO, protein; PS, polysaccharide; ROS, reactive oxygen species; SBR, sequencing batch reactor; SEM, scanning electron microscope; SVI, sludge volume index; TB-EPS, tightly bound EPS; TOC, total organic carbon; WWTP, wastewater treatment plant.

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some types of NPs such as TiO_2, ZnO, CeO_2, and Ag could decrease the population of the microbial community and disturb the microbial diversity in activated sludge systems (García et al., 2012; Zheng et al., 2011). Most of the studies so far have focused on the effect of NPs on the microbial growth activity (Choi et al., 2008; Liang et al., 2010; Zhang et al., 2014), change in the bacterial community structure (Sun et al., 2013; Yang et al., 2014), and decrease in the chemical oxygen demand (COD) and nitrogen/phosphorus contents (Hou et al., 2012; Li et al., 2013). However, the effect of NPs on the physicochemical stability of activated sludge has been seldom reported (Yang et al., 2013).

The physicochemical stability of activated sludge flocs plays an important role in the performance of wastewater treatment systems (Li and Yang, 2007; Ye et al., 2011). Activated sludge generally exists as flocs, which are suspended microbial aggregates containing microorganisms and organic/inorganic compounds (Biggs and Lant, 2000). Flocculating ability, sludge settling, and sludge dewatering are the regularly monitored physicochemical properties of activated sludge (Jin et al., 2003; Wilén et al., 2003). In particular, extracellular polymeric substances (EPS), which are the major components of the activated sludge, act as a gel-like matrix that binds the cells together to form sludge flocs (Sheng et al., 2010). There are two types of EPS, namely the loosely bound EPS (LB-EPS) and the tightly bound EPS (TB-EPS) (Li et al., 2014). The production and composition of the EPS can significantly affect biomass granulation (Caudan et al., 2012), flocculation (Li and Yang, 2007), and the structure of the bioaggregates (Seviour et al., 2012). It is reasonable to assume that the EPS play a leading role in flocculation, settling, and dewatering properties of the flocs. In addition, EPS are usually thought to protect the inner microorganisms from the harsh external environmental conditions such as exposure to heavy metals or chemicals (Ma et al., 2013; Sheng et al., 2013). Thus, the EPS may determine the physicochemical properties and give the floc its structural and functional integrity (Niu et al., 2013; Sheng et al., 2010). Therefore, it is very important to explore the nature and composition of the EPS in activated sludge in the presence of NPs.

In previous studies, CuO NPs were chosen as model NPs, since they are widely used in antibactericide coatings, biomedicines, and toothpastes owing to their unique physicochemical properties such as enhanced magnetic, electrical, and optical features (Applerot et al., 2012; Zhao et al., 2013). However, the effects of CuO NPs on the physicochemical properties of the sludge and the production and composition of the EPS have never been reported.

For these reasons, the effect of CuO NPs on the physicochemical properties (such as flocculating ability, settleability, and dewaterability) of activated sludge, and in particular, on the EPS production and composition, were investigated. In addition, possible mechanisms explaining the changes in the physicochemical characteristics of the sludge induced by different concentrations of CuO NPs were explored.

2. Methods

2.1. CuO nanoparticles and activated sludge

Powdered nanosized CuO NPs (with a mean diameter of 92 ± 12 nm; see Supplementary material, Table S1 for detailed information on the properties) were purchased from Sigma–Aldrich (St. Louis, MO). A scanning electron microscope (SEM) image of the CuO NPs was obtained using a Hitachi S-4800 SEM to get a visual idea of their shape (Fig. S1, Supplementary material). In this study, a stock suspension of the CuO NPs was prepared by adding 0.3 g of the CuO NPs to 1 L of Milli-Q deionized water (pH 6.9 ± 0.1). Subsequently, the stock suspension was ultrasonicated (20 °C, 250 W, 40 kHz) for 30 min before diluting it to the desired exposure concentration (Zhao et al., 2013). The particle size distribution and the zeta potential of the CuO NPs were measured using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK).

The flocculent sludge samples were collected from the sedimentation tank of the Jiangning Municipal Wastewater Treatment Plant (Nanjing, China). The flocculent sludge (5 g of mixed liquor volatile suspended solids (MLVSS)/L) was first acclimatized to the synthetic wastewater in a parent sequencing batch reactor (SBR) for about two months until stable performance was achieved (details are provided in the Supplementary material). The synthetic wastewater contained 1060 mg/L of glucose, 90 mg/L of NH_4Cl, 400 mg/L of $K_2HPO_4{\cdot}3H_2O$, 360 mg/L of $KH_2PO_4{\cdot}2H_2O$, 12 mg/L of $CaCl_2{\cdot}2H_2O$, 50 mg/L of $MgSO_4$, $MgSO_4$ 50 mg/L of $MgSO_4$, 8 mg/L of $MnSO_4{\cdot}H_2O$, 2 mg/L of $ZnSO_4{\cdot}7H_2O$, 1 mg/L of $CuSO_4{\cdot}5H_2O$, and 1 mg/L of $(NH_4)_6MoO_{24}{\cdot}4H_2O$. The temperature was maintained at about 20–22 °C and the pH was controlled at 7.0–7.5.

2.2. Experimental design

In order to investigate the effect of the CuO NPs on the stability of the activated sludge, three different concentrations of CuO NPs were examined in batch experiments, which were conducted in a series of reactors, each having a working volume of 250 mL. The stock suspension containing the CuO NPs was diluted to concentrations of 5 mg/L, 20 mg/L, and 50 mg/L and added into the reactors. The activated sludge, withdrawn from the parent SBR reactor, was inoculated into each reactor along with approximately 3 g of MLVSS/L. The mixtures were then equilibrated in a rotary shaker, with a rotation speed of 160 rpm (20 °C) for 12 h. Control tests in the absence of the CuO NPs were also conducted for comparison and all the experiments were carried out in triplicate. After exposure to the CuO NPs, the sludge samples were withdrawn and the amount and the composition of the EPS in the activated sludge were measured.

2.3. EPS extraction and analysis

Extraction of the EPS was carried out using a process involving centrifugation, sonication, and thermal extraction (Ye et al., 2011). At the end of the batch experiments, 2000*g* of the activated sludge was harvested by centrifugation for 10 min. The sludge pellet was re-suspended in a 0.05% (w/w) NaCl solution, and sonicated at 20 kHz for 2 min. The suspension was then centrifuged at 8000*g* for 20 min and the liquid was collected carefully for measuring the LB-EPS. The residual sludge pellet left in the centrifuge tube was re-suspended in a 0.05% (w/w) NaCl solution and heated at 70 °C for 30 min. These conditions have been previously shown to provide a relatively high extraction efficiency and low cell lysis (D'Abzac et al., 2010). The suspension was centrifuged at 12,000*g* for 20 min, and the supernatant was the TB-EPS fraction. Finally, all the EPS fractions were filtered through 0.45 μm acetate cellulose membranes.

Both the LB-EPS and TB-EPS extractions were analyzed for total organic carbon (TOC), proteins (PRO), and polysaccharides (PS). The TOC was measured using a TOC analyzer (Liqui TOC II, Elementar, Germany), using the combustion-infrared method. The PRO and PS were analyzed using the modified Lowry method and the anthrone–sulfuric acid method respectively, according to reports in the literature (Li and Yang, 2007).

2.4. Fourier transform-infrared spectroscopy (FT-IR)

FT-IR analysis was used to obtain information on the major functional groups in EPS and their interaction with Cu^{2+}/CuO NPs (Sheng et al., 2013). Prior to the analysis, the wet samples were