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Research article

Long-term effects of cupric oxide nanoparticles (CuO NPs) on the performance, microbial community and enzymatic activity of activated sludge in a sequencing batch reactor

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ABSTRACT

The long-term effects of cupric oxide nanoparticles (CuO NPs) on the performance, microbial activity and microbial community of activated sludge were investigated in a sequencing batch reactor (SBR). The SBR performance had no evident change at 0–10 mg/L CuO NPs, whereas the CuO NPs concentration at 30–60 mg/L affected the COD, NH_4^+-N and soluble orthophosphate (SOP) removal, nitrogen and phosphorus removal rate and microbial enzymatic activity of activated sludge. Some CuO NPs might be absorbed on the surface of activated sludge or penetrate the microbial cytomembrane into the microbial cell interior of activated sludge. Compared to 0 mg/L CuO NPs, the reactive oxygen species (ROS) production and lactate dehydrogenase (LDH) release increased by 43.6% and 56.4% at 60 mg/L CuO NPs, respectively. The variations of ROS production and LDH release demonstrated that CuO NPs could induce the toxicity towards the microorganisms and destroy the integrity of microbial cytomembrane in the activated sludge. High throughput sequencing of 16S rDNA indicated that CuO NPs could evidently impact on the microbial richness, diversity and composition of activated sludge in the SBR.

1. Introduction

Cupric oxide nanoparticles (CuO NPs) have been widely utilized in gas sensors, wood preservation, antimicrobial textiles, batteries, catalytic processes, marine antifouling, plastics and metallic coating (Adam et al., 2015; Heinlaan et al., 2008; Mortimer et al., 2010). The negative impacts of CuO NPs on organism and human have attracted extensive attention due to their nanosize and specific properties. CuO NPs have shown evident toxicity to bacteria (Heinlaan et al., 2008), algae (Aruoja et al., 2009), yeast (Kasemets et al., 2009), protozoa (Mortimer et al., 2010), mammalian cells (Bondarenko et al., 2013) and *Daphnia magna* (Mwaanga et al., 2014). Xu et al. (2015) reported that the existence of CuO NPs in the flooded paddy soil could reduce the composition and diversity of microbial community. Some researches pointed out that CuO NPs could lead to significant toxicity to human lung epithelial cells and human alveolar epithelial cells through DNA damages (Wang et al., 2012; Moschini et al., 2013).

CuO NPs are inevitably released into industrial and municipal wastewater owing to the rapid increase of their production and application (Ganesh et al., 2010), and it is very necessary to investigate the potential effects of CuO NPs on different biological wastewater treatment systems. Liu and Wang (2012) evaluated the short-term influence of CuO NPs on the COD removal, ammonia oxidization, endogenous respiration and nitrite oxidization of activated sludge by the respiration rate measurement. Luna-delRisco et al. (2011) indicated that the biogas production during anaerobic digestion of cattle manure could be obviously inhibited at the CuO NPs concentration over 15 mg/L. Otero-González et al. (2014) pointed out that low CuO NPs concentration only caused a slight inhibition to methanogenesis under short-term exposure in high-rate anaerobic bioreactors, while the long-term exposure could cause severe toxicity and reduce the acetoclastic

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methanogenic activity by more than 85%. Hou et al. (2015) illustrated that the presence of CuO NPs could impact on the flocculating ability of activated sludge and the composition of extracellular polymeric substances from activated sludge. Chen et al. (2012) reported the long-term effects of CuO NPs on the wastewater biological nutrient removal and N_2O generation in the activated sludge process. Hou et al. (2016a,b) also investigated the long-term effects of CuO NPs on nitrogen removal and the surface physicochemical properties of biofilms in the sequencing batch biofilm reactor. Ünşar et al. (2016) reported the long and short term impacts of CuO NPs on the anaerobic digestion of waste activated sludge. As far as we know, little information is found in evaluating the long-term effects of CuO NPs on the performance, microbial enzymatic activity and microbial community of activated sludge and analyzing the toxicity of CuO NPs to activated sludge from a SBR.

The aims of this study were (i) to investigate the long-term effects of CuO NPs on the nitrogen and phosphorus removal, microbial activity and microbial enzymatic activity of activated sludge in the SBR; (ii) to evaluate the variations of microbial richness and diversity in the activated sludge at different CuO NPs concentrations through high-throughput sequencing; (iii) to analyze the toxicity of CuO NPs to activated sludge and the fate of CuO NPs in the SBR.

2. Materials and methods

2.1. Preparation of CuO-NPs suspension

CuO NPs was obtained from Beijing DK Nano Technology Co., Ltd. (Beijing, China), which had the particle diameters of about 40 nm. The suspension at 500 mg/L CuO NPs was prepared as follows (Keller et al., 2010): Firstly, 0.5 g CuO NPs was put into a 1 L volumetric flask, and then the volume of CuO NPs suspension was fixed to 1 L by adding Milli-Q water to the volumetric flask. Secondly, the CuO NPs suspension was mixed by 1 h ultrasound. The average hydrodynamic diameter, polydispersity index and particle size peak of CuO NPs in the suspension were measured to be 588.3 nm, 0.611 and 255.6 nm, respectively.

2.2. Experimental set-up and synthetic wastewater

A laboratory-scale SBR used in our study was 14 cm inner diameter and 50 cm effective height, which had an effective volume of 7.69 L. The temperature in our laboratory has been controlled by the air-condition in summer and the central heating in winter, and the SBR operated at about 25 °C during the whole operational period. The seed sludge was obtained from the recycled sludge of the secondary clarifier in Licunhe municipal wastewater treatment plant in Qingdao City, China. The recycled sludge was firstly settled for 30 min and then decanted the supernatant. The settled sludge in an incubator was transported to the laboratory as quickly. After the settled sludge was mixed with synthetic wastewater in the SBR, the initial mixed liquor suspended sludge (MLSS) of SBR was about 3500 mg/L. An influent pump introduced the synthetic wastewater into the SBR, and the effluent was discharged through a solenoid valve at a height of 25 cm from the bottom. The volume exchange ratio of SBR was 50% at each circle. The SBR operational procedure of each cycle included influent stage (0.1 h), anoxic stage (2.4 h), aerobic stage (4.0 h), settling stage (1.3 h), and discharge stage (0.20 h). A magnetic stirrer was used to mix the synthetic wastewater and activated sludge at the anoxic period, and air was introduced by the air diffusers at the aerobic period. According to the previous reports (Zheng et al., 2011; Puay et al., 2015), the components of synthetic wastewater with a slight adjustment were shown as follows (mg/L): CH_3COONa (510), $NaHCO_3$ (120), NH_4Cl (82), KH_2PO_4 (53), $ZnSO_4{\cdot}7H_2O$ (0.12), K_2HPO_4 (16), $MnCl_2{\cdot}4H_2O$ (0.12), $Na_2MoO_4{\cdot}2H_2O$ (0.06), $CuSO_4{\cdot}5H_2O$ (0.03), KI (0.03), H_3BO_3 (0.15), $CoCl_2{\cdot}6H_2O$ (0.15), and $FeCl_3{\cdot}6H_2O$ (1.5). The concentrations of COD, soluble orthophosphate (SOP), and NH_4^+-N in the synthetic wastewater were approximately 400, 10, and 25 mg/L, respectively.

2.3. Analytical methods

The determinations of COD, NH_4^+-N, NO_3^--N, SOP, NO_2^--N and MLSS were carried out according to the standard methods (APHA, 1998). The Cu content was measured by a flame atomic adsorption spectrometer (SOLLAR M6, Thermo, USA). The measurements of specific ammonia oxidation rate (SAOR), specific nitrite oxidation rate (SNOR), specific oxygen utilization rate (SOUR), specific nitrate reduction rate (SNRR), specific nitrite reduction rate (SNIRR), specific phosphorus release rate (SPRR), and specific phosphorus uptake rate (SPUR) of activated sludge were performed according to Wang et al. (2016a,b). The dehydrogenase (DHA) activity was measured by using triphenyl tetrazolium chloride (TTC) as hydrogen acceptor (Klapwuk et al., 1974). The reactive oxygen species (ROS) production of activated sludge was measured by the dichlorodihydrofluorescein (DCF) assay method (Zheng et al., 2011). The lactate dehydrogenases (LDH) were measured by a LDH kit (Beyotime Biotechnology, Jiangsu, China). The nitrite oxidoreductase (NOR), ammonia monooxygenase (AMO), nitrite reductase (NIR), nitrate reductase (NR), polyphosphate kinase (PPK), and exopolyphosphatase (PPX) of activated sludge were measured according to previous report (Wang et al. et al., 2016a,b). The surface distribution and intracellular distribution of activated sludge were observed by a scanning electron microscopy (SEM, S-4800, Hitachi) and a transmission electron microscopy (TEM, H-7650, Hitachi), respectively. The microbial communities of SBR at different CuO NPs concentrations were analyzed by an Illumina MiSeq platform of Novogene (Beijing, China) according to Gao et al. (2014).

2.4. Statistical analysis

Some assays were conducted in triplicate and the results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was used to test the significance of results, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Performance evaluation of SBR at different CuO NPs concentrations

The COD, nitrogen and phosphorus removal, SOUR and nitrogen and phosphorus removal rate in the SBR were investigated at different CuO NPs concentrations. As shown in Fig. 1, the COD removal efficiency kept a relatively steady value at 91.26 ± 0.99% at 0–10 mg/L CuO NPs. However, the COD removal efficiency decreased to 89.48 ± 0.54% at 30 mg/L CuO NPs and 87.61 ± 0.81% at 60 mg/L CuO NPs, suggesting that high CuO NPs concentration could lead to a decrease in the COD removal. No evident change in the NH_4^+-N removal was found at 0–30 mg/L CuO NPs, whereas the NH_4^+-N removal efficiency decreased from 98.3 ± 1.4% at 30 mg/L CuO NPs to 96.9 ± 0.6% at 60 mg/L CuO NPs (Fig. 1b). As shown in Fig. 1c, the effluent NO_2^--N concentration increased from 0.07 ± 0.09 at 0 mg/L CuO NPs to 1.84 ± 0.14 mg/L at 60 mg/L CuO NPs, indicating that CuO NPs could lead to the accumulation of NO_2^--N in the SBR. The effluent NO_3^--N concentration had no evident variation at 0–5 mg/L CuO NPs, and the effluent NO_3^--N