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Effect of copper oxide nanoparticles on the ammonia removal and microbial community of partial nitrification process

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HIGHLIGHTS

- Short- and long-term effects of CuO NPs on PN process was investigated.
- CuO NPs with low concentration ($\leq$5 mg L^{-1}) was profitable for PN process.
- The suppression threshold on AOB of short-term CuO NPs was 30 mg L^{-1}.
- The suppression threshold on AOB of long-term CuO NPs was 10 mg L^{-1}.
- CuO NPs ($\geq$10 mg L^{-1}) led to microorganism death and EPS decrease.

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ABSTRACT

Autotrophic nitrogen removal is an innovative and economical nitrogen removal technology, in which partial nitrification (PN) is the key component. It is necessary to clear the impact of CuO nanoparticles (CuO NPs) on PN process since the wide application increased their opportunity for entering into wastewater. In this study, the short-term and long-term effects of CuO NPs on ammonia removal, extracellular polymeric substance (EPS) production and microbial community were investigated. Results suggested that CuO NPs in 1 mg L^{-1} had slight effect on PN, while that in 3–10 mg L^{-1} enhanced ammonia oxidation in the short-term exposure. In the long-term exposure experiment, CuO NPs with low concentration ($\leq$5 mg L^{-1}) improved both the bioactivity and relative abundance of ammonia-oxidizing bacteria (AOB). The suppression threshold on PN process of CuO NPs in short-term exposure was 30 mg L^{-1}, while that in long-term exposure decreased to 10 mg L^{-1} due to the adsorption and accumulation of NPs. *Nitrosomonas* was the predominant AOB in PN reactors, it showed better tolerance to CuO NPs and performed the best performance when CuO NPs was 5 mg L^{-1}. Long-term exposure within CuO NPs led to organisms death and resulted in the decrease of EPS.

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1. Introduction

Nowadays, most organic compounds in wastewater are expected be converted to biogas by adopting anaerobic treatment process, which is feasible with the present state of the art [1]. As a result, many wastewaters do not contain sufficient amounts of biodegradable carbon for nitrogen removal via the traditional nitrification-denitrification process. Therefore, the autotrophic nitrogen removal process based on partial nitrification (PN) and Anammox is becoming as one alternative technology and attracts increasing attention, since it could remove nitrogen with no organic carbon and less oxygen consumption [2]. In autotrophic nitrogen removal process, partial ammonia was oxidized to nitrite in PN stage, then the effluent of PN stage that contained remained ammonia and nitrite were converted to N_2 in Anammox stage. One thing to note is the occurrence of Anammox requires influent with a certain proportion of nitrite to ammonia (about 1.32), therefore, the PN stage which undertakes partial oxidation of ammonia to nitrite is critical and indispensable. In PN process, the oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB) should be enhanced while the oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB) should be suppressed [3]. In consequence, profitable bioactivity of AOB was essential for a stable and high-rate PN process.

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AOB bioactivity was influenced by many factors in the wastewater, such as heavy metals, antibiotics, salinity and sulfides [4–8], from which it could be concluded that AOB was sensitive to the wastewater quality. 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 [9]. Some reports suggested that the wide use of nanoparticles (NPs) has inevitably caused their release into the environment, especially into wastewater treatment systems [10,11]. The adsorbed and aggregated NPs in wastewater treatment system may have adverse effects on the microbial bioactivity and disrupt bioreactor performance, which may also bring threats to the effluent quality.

In recent years, cupric oxide nanoparticles (CuO NPs) has been widely utilized in catalysis processes, chemical sensors, antimicrobial textiles and marine antifouling, owing to their optical, catalytic, antibacterial, and semiconductor properties [12,13]. Additionally, CuO NPs are also generated in large amounts as a by-product of chemical mechanical polishing in the semiconductor industry. As a result, CuO NPs are inevitably released into industrial and municipal wastewater [12,14]. It was reported that the concentrations of copper in semiconductor industry effluents can reach up to 100 mg L^{-1}, with nearly half of this amount present as CuO NPs [15]. It has been reported about the adverse effects of CuO NPs on aquatic organisms [16], algae of normal ecosystem [17], human health [18], cell viability of activated sludge [10], as well as cause cytotoxicity and DNA damage [19]. Considering the widely use and toxic effects on many kinds of organisms, it is very necessary to investigate the potential effects of CuO NPs on biological wastewater treatment systems. Previous studies have proved the remarkable influences of CuO NPs on some wastewater treatment systems, such as methane production system [20,21], and conventional activated sludge system [22,23]. However, limited study focused on the efficient and economical autotrophic nitrogen removal process. As the key component of autotrophic nitrogen removal technology, it was essential to clarify the potential impact of CuO NPs on PN process.

The main goal of this study was (i) to investigate the short and long-term effects of CuO NPs on PN performance, (ii) to determine the suppression threshold of CuO NPs on AOB, and (iii) to determine the variation of microbial characteristics under CuO NPs effects.

2. Material and methods

2.1. Sludge culturing and CuO NPs suspension

PN sludge used in this study was obtained from one membrane bioreactor (MBR), which was operated as PN process for more than one year. The ammonia removal rate, nitrite accumulation rate (NAR) of the MBR was about 0.6 kg m^{-3} d^{-1} and 99%, the mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) of the sludge for this experiment was 3.7 and 2.7 g L^{-1}, respectively. The synthetic wastewater contained 0.985 g L^{-1} $(NH_4)_2SO_4$ as ammonia source and 2.685 g L^{-1} $NaHCO_3$ as alkalinity source. The CuO NPs were purchased from Aladdin Company, the average diameter of the particles size was 40 nm. The scanning electron microscopy image and X-ray diffraction spectra of CuO NPs were shown as Supplemented material 1 and 2. CuO NPs stock solution (1 g L^{-1}) was concocted by adding 1.0 g CuO NPs to 1.0 L distilled water. Stock dispersion was sonicated (250 W, 40 kHz) for 1 h to break down aggregates before being diluted to exposure concentrations. The NPs suspension was freshly prepared based on above-mentioned procedures just prior to the addition of them into the reactor.

2.2. Experimental set-up

Batch experiments were conducted in six flakes with 100 mL effective volume, to study the short-term effect (3 h) of CuO NPs. The experimental sludge obtained from the stable MBR was washed by the synthetic wastewater for more than three times, to make the same water quality in the six flakes. Then CuO NPs solution was added to each flake filled with experimental sludge, making the final concentration to 0, 1, 3, 5, 10 and 30 mg L^{-1}, respectively. The influent ammonia, initial pH, DO, and T in short-term exposure experiment were about 200 mg L^{-1}, 7.6, 0.2 mg L^{-1}, 25–26 °C, respectively. The six flakes were operated for a cycle within the exposure of CuO NPs (3 h), water sample was taken out every 30 min, to detect the nitrogen components. During the exposure period, the flakes were set up in triplicate, the average result was used to consider the short-term effect of CuO NPs.

For the long-term exposure (60 days) of CuO NPs, six sequencing batch reactors with 1 L effective volume were operated for 60 days, named P0, P1, P2, P3, P4, P5, respectively. 1 L sludge taken from the MBR was seeded to each reactor for the long-term experiment, to make the initial MLSS and MLVSS as 3.7 and 2.7 g L^{-1}, respectively. P0 without CuO NPs addition was set as the controlled reactor, while P1, P2, P3, P4 and P5 were operated as the experimental reactors, with the addition of CuO NPs of 1, 3, 5, 10, 30 mg L^{-1}, respectively. For the six reactors, three cycles were operated in one day, each cycle contained: feeding, 5 min; reacting, 3 h; settling, 0.5 h; draining, 5 min. For each cycle, the sludge was washed by distilled water for three times before CuO NPs and synthetic wastewater were added. The variations of nitrogen components in the second cycle were daily measured, to reveal the long-term effects of CuO NPs. Additionally, at the end of the long-term exposure experiment, the sludge sample of each reactor was taken to analyze the extracellular polymeric substance (EPS) and microbial community variation. The operational conditions of the reactors during the long-term exposure experiment were shown as Table 1.

2.3. Analytical methods

Concentrations of NH_4^+-N and NO_2^--N were daily measured using different colorimetric methods and NO_3^--N was analyzed using ultraviolet spectrophotometric method. The temperature, DO and pH were detected using portable instruments with specific probes (WTW, Germany). The ammonia removal efficiency (ARE), NAR and specific ammonia oxidation rate (SAOR) were calculated as Eqs. (1)–(3), respectively. The t in Eq. (3) represents the reaction time in one cycle.

$$ARE = \frac{[NH_4^+]_{Inf.} - [NH_4^+]_{Eff.}}{[NH_4^+]_{Inf.}} \times 100\% \quad (1)$$

$$NAR = \frac{[NO_2^-]_{eff.}}{[NO_2^-]_{eff.} + [NO_3^-]_{eff.}} \times 100\% \quad (2)$$

$$SAOR = \frac{[NH_4^+]_{Inf.} - [NH_4^+]_{Eff.}}{t \times MLVSS} \quad (3)$$

2.4. High-throughput pyrosequencing and phylogenetic assignment

The DNA was qualified by Qubit2.0 DNA detection kit (Sangon, China). The qualified DNA was high-throughput pyrosequencing sequenced by Novogene Company. The PCR primers were V3-V4 universe primers 341F/805R (341F: CCTACGGGNGGCWGCAG; 805R: GACTACHVGGGTATCTAATCC). The high-throughput pyrose-

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