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Chronic responses of aerobic granules to zinc oxide nanoparticles in a sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal

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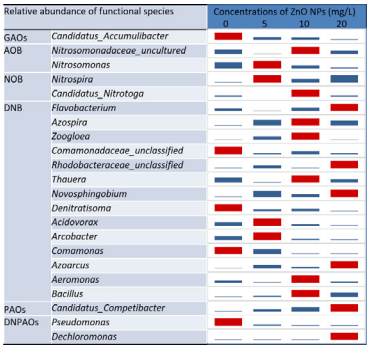
HIGHLIGHTS

- Chronic responses of aerobic granules SBR to ZnO NPs was assessed for the first time.
- ZnO NPs stimulated carbon uptake, inhibited nitrogen removal and did not affect phosphorus removal.
- Up to 20 mg/L ZnO NPs significantly reduced bacterial diversity and richness.
- Both relative abundances and spatial distribution of microbial community shifted after exposure to ZnO NPs.
- Introduction of ZnO NPs led to decrease of GAOs and AOB, and accumulation of NOB, DNB, PAOs and DNPAOs.

GRAPHICAL ABSTRACT



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ABSTRACT

The reactor performance, granules characteristics and microbial population dynamics were investigated to assess the chronic responses of aerobic granules to zinc oxide nanoparticles (ZnO NPs) of 0, 5, 10 and 20 mg/L for a period of 180 days. The results showed that ZnO NPs stimulated COD removal, whereas caused inhibition to both nitrification and denitrification. However, biological phosphorus removal remained effective and stable. Introduction of ZnO NPs sharply decreased the respiration of granules, while did not change the settleability. Both content of extracellular polymeric substances (EPS) and the ratio of protein to polysaccharides (PN/PS) rose significantly. MiSeq pyrosequencing was employed to explore the microbial population dynamics. Results demonstrated that up to 20 mg/L reduced the alpha-diversity of bacterial communities. Finally, phylogenetic classification of the dominant functional species involved in biological nutrients removal were identified to assess the effects of ZnO NPs to aerobic granules from the molecular level.

1. Introduction

Due to the increasing production and wide application of nanomaterials, it is inevitable that nanoparticles can be leaked into the environment, ending up in aquatic systems (Kiser et al., 2009). Wastewater treatment plants (WWTPs) are important barriers to

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prevent nanoparticles from directly entering into the environment (Unsar et al., 2016). Kiser et al. (2009) and Gottschalk et al. (2009) have reported detection of nanoparticles (NPs) in the WWTPs performing biological wastewater treatment. Zinc oxide nanoparticles (ZnO NPs) have raised attention due to their widespread medical, industrial and military applications (Mu and Chen, 2011), which has inevitably led to their release into the environment. Jones et al. (2008) reported that ZnO NPs concentrations of 100 μg/L in water to the mg/kg in soil were detected in Britain. Studies have also predicted the potential threats of ZnO NPs released into water to the microbes, including inactivating impacts on bacteria (Brayner et al., 2006), inhibitory effects on key enzyme (Hou et al., 2014) and transformation of microbial community structures (Mu and Chen, 2011). Some researchers have focused on the environmental impacts of ZnO NPs on activated sludge (Zheng et al., 2011), biofilm (Xu et al., 2016), pure strain of microbes (Adams et al., 2006) and anaerobic granular sludge (Mu et al., 2012). Our previous study investigated the shock loading of ZnO NPs on aerobic granules SBR for wastewater treatment (He et al., 2017). However, little is known about the chronic eco-toxicity of ZnO NPs to the aerobic granular sludge (AGS) performing simultaneous nitrogen and phosphorus removal.

The key microorganisms involved in biological nitrogen and phosphorus removal contains ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), denitrifying bacteria (DNB), phosphorus accumulating organisms (PAOs) and denitrifying PAOs (DNPAOs) (Oehmen et al., 2010). Biological nitrogen removal is achieved by nitrification and denitrification processes, where AOB and NOB are responsible for nitrification process, and DNB conducts denitrification. Both PAOs and DNPAOs can perform biological phosphorus removal, while DNPAOs can simultaneously remove nitrogen and phosphorus via nitrite (NO_2^--N) or nitrate (NO_3^--N) (He et al., 2016a; Miao et al., 2016). Besides, the competition between heterotrophic glycogen accumulating organisms (GAOs) and PAOs/DNPAOs largely affect the biological nutrients removal when carbon source is limited (Kishida et al., 2006; Wang et al., 2015b). Exploring the relative abundances and distributions helps demonstrate the inner mechanism for toxicity to biological nitrogen and phosphorus removal (Miao et al., 2016). To date, no previous research has been conducted to reveal the effect of ZnO NPs on these functional organisms for biological nutrients removal in SBR.

Therefore, the major purpose of the present study is to assess the long-term effect of ZnO NPs on the aerobic granules SBR in terms of reactor performance, physical characteristics, bacterial population dynamics. Phylogenetic classification of the dominant functional species responsible for biological nitrogen and phosphorus removal were identified to reveal the mechanism for toxicity of ZnO NPs to aerobic granules from the molecular level. This work might contribute to the detailed information on the chronic toxicity of ZnO NPs on aerobic granules SBR.

2. Materials and methods

2.1. Experimental setup

An aerobic granular sludge SBR with a diameter of 100, height of 500 mm and an effective volume of 3.6 L was operated on an anaerobic/oxic/anoxic (AOA) mode in the present study (Fig. S1) and configured as previous work assessing the impact of shock loading (He et al., 2017), so as the preparation of synthetic ZnO NPs and 0.5 M stock solution. 5, 10 and 20 mg/L ZnO NPs were used at different operational stages (i.e., four phases with increasing concentrations of ZnO NPs from 0, 5, 10 to 20 mg/L and each with a period of 45 days, total up to 180 days). The compositions of the synthetic wastewater used in present work was according to the previous research (He et al., 2016a, 2017), briefly, chemical oxygen demand (COD) 183 mg/L, ammonia nitrogen (NH_4^+-N) 17 mg/L, total inorganic nitrogen (TIN) 19 mg/L and total phosphorus (TP) 3 mg/L on average. Sodium acetate (NaAc) was used as the sole carbon source for the present study.

2.2. MiSeq pyrosequencing

Four aerobic granular sludge samples were collected at the end of each operational stage (day 45, 90, 135 and 180, denoted as Z1, Z2, Z3 and Z4, respectively) under the ZnO NPs concentrations of 0, 5, 10 and 20 mg/L. DNA extraction, PCR amplification and pyrosequencing of the V3-4 region of the 16S rRNA gene were conducted sequentially using the primer sets 338F (5′-ACTCCTACGGGAGG CAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) as the procedures by previous studies (He et al., 2016b; Wang et al., 2015a). Sequencing of the targeted gene of the collected samples was conducted using the Illumina MiSeq platform (PE30, CA, USA) following the manufacturer's instructions. The bioinformatics analysis was run as previous work (He et al., 2016b; Wang et al., 2015a).

2.3. Analytical methods

The COD, nitrogen (including NH_4^+-N, nitrate (NO_3^--N), nitrite (NO_2^--N)), TP, MLSS, sludge volume index at 5 min (SVI_5) were measured according to the standard methods (APHA, 2005). TIN was regarded as the sum of NH_4^+-N, NO_3^--N, NO_2^--N (Long et al., 2014). The pH and DO were measured using a pH meter (pHS-25, pHS-25, Shanghai Leici Instrument Factory, China) and DO meter (YSI5000, YSI, Yellow Springs, Ohio, USA). Oxygen uptake rate (OUR), specific oxygen uptake rate (SOUR) were determined as He et al. (2017). EPS were extracted with a modified heat extraction method by Yang et al. (2014). Protein (PN) content was determined by a modified Lowry method and polysaccharides (PS) content was analyzed using a phenol-sulfuric acid method (Long et al., 2014). EPS was regarded as the sum of PN and PS.

3. Results and discussion

3.1. Reactor performance

The aerobic granules SBR performing simultaneous carbon, nitrogen and phosphorus removal was run for 180 days, with increasing ZnO NPs concentrations of 0, 5, 10 and 20 mg/L for a period of 45 days each, respectively. Fig. 1 illustrated the removal performance for COD, NH_4^+-N, TIN and TP during the whole operational periods. As shown in Fig. 1, the COD removal efficiency increased slightly with the addition of 5–20 mg/L ZnO NPs from 89.73 to 99.35%. In contrast, both nitrogen (including NH_4^+-N and TIN) and phosphorus (TP) was inhibited by certain degrees under long-term exposure to ZnO NPs. As a result, TP removal rates changed slightly from 98.44 to 88.56%, while NH_4^+-N and TIN removal efficiencies dropped by 24.75 and 36.14%, respectively. Our previous research studying the shock loading of ZnO NPs on aerobic granular SBR showed similar effects on carbon, nitrogen and phosphorus removal (He et al., 2017). Hou et al. (2013) found that up to 5 mg/L ZnO NPs did not reduce COD removal, but inhibited the NH_4^+-N removal. Previous researches also reported inhibitory effects of ZnO NPs to COD, NH_4^+-N, TIN and TP removal (Wang et al., 2016b; Zheng et al., 2011).

To further reveal the mechanisms of ZnO NPs on simultaneous carbon, nitrogen and phosphorus removal, cycle performances under exposure to different concentrations of ZnO NPs were con-