



## Short Communication

## Insight into the impact of ZnO nanoparticles on aerobic granular sludge under shock loading



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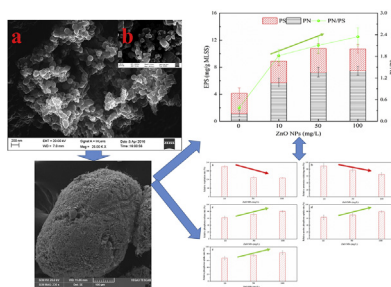
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## HIGHLIGHTS

- Environmental risks of ZnO NPs to aerobic granular sludge was explored for the first time.
- ZnO NPs caused acute toxicity to both nitrification and denitrification processes.
- The COD and phosphorus removal remained unaffected by exposure to 10–100 mg/L ZnO NPs.
- The activities of respiration was improved while both nitrogen and phosphorus rates were inhibited.
- The over-produced EPS (mainly PN) helped relieve the toxicity of ZnO NPs.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The increasing use of zinc oxide nanoparticles (ZnO NPs) has raised concerns about the environmental threats to the wastewater treatment systems. Shock loading of 10, 50 and 100 mg/L ZnO NPs was conducted to evaluate impacts on reactor performance, microbial activities and extracellular polymeric substances (EPS) in parent aerobic/oxic/anoxic (A/O/A) granular sequencing batch reactors (SBRs). The results showed that ZnO NPs caused inhibition to nitrogen transformations due to acute toxicity to nitrification and denitrification. However, phosphorus removal remained unaffected by the exposure to ZnO NPs. Besides, ZnO NPs significantly enhanced the oxygen respiration rate and caused acute toxicity to ammonia oxidizing rate (10.40–35.21%), phosphorus release rate (37.79–19.80%), aerobic phosphorus uptake rate (36.95–20.69%) and total phosphorus uptake rate (32.77–16.91%) of aerobic granules. ZnO NPs stimulated the secretion of EPS, especially the content of protein (PN), which could relieve the toxicity of ZnO NPs.

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## 1. Introduction

With the rapid development of nanotechnology, nanoparticles have been widely used in numerous industrial products like cosmetics, pigments, semiconductors and biomedicines (Gottschalk

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et al., 2009). It is inevitable that they can be leaked into the environment, ending up in aquatic systems. Wastewater treatment plants (WWTPs) are important barriers to prevent nanoparticles from directly entering into the environment (Unsar et al., 2016). In recent years, nanoparticles have been detected in WWTPs performing biological wastewater treatment (Kiser et al., 2009). Therefore, it is of urgent concern to explore the potential influence of nanoparticles on biological treatment processes. Zinc oxide nanoparticles (ZnO NPs) have received increasing interest 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 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 the 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). Kasemets et al. (2009) found that the release of  $Zn^{2+}$  from ZnO NPs was an important contributor to the ecotoxicity and that the ZnO NPs induced more reactive oxygen species (ROS) production, which caused inhibitions on enzyme activities (Zheng et al., 2011). Although some researches have been conducted to investigate the environmental impacts of ZnO NPs on activated sludge (Zheng et al., 2011), biofilm (Xu et al., 2016) and anaerobic granules (Mu et al., 2012), with almost all studies on the biological nitrogen removal, little is known about the eco-toxicity of ZnO NPs to the aerobic granular sludge (AGS) performing simultaneous nitrogen and phosphorus removal.

AGS as the aggregation of microbes shows different characteristics compared with activated sludge or biofilm. Therefore, the responses to the presence of ZnO NPs may differ due to its layered structure and other unique physicochemical attributes (Quan et al., 2015). It has been well known that AGS can secrete extracellular polymeric substances (EPS) to protect from the inhibitory effects of toxic matters including phenol, metal ions and NPs and so on (Mu et al., 2012; Quan et al., 2015; Shi et al., 2013; Xu et al., 2016; Zheng et al., 2011). To date, the shock loading of ZnO NPs on the reactor performance and microbial activities of AGS still remain unknown.

Therefore, the major purpose of the present study is to assess the shock loading of ZnO NPs on the biological treatment processes in an AGS sequencing batch reactor (SBR) based on the nitrogen and phosphorus removal, microbial activities and EPS production. This work might contribute to the detailed information on the acute impacts of ZnO NPs on AGS.

## 2. Materials and methods

### 2.1. Preparation of ZnO NPs

ZnO NPs were prepared using the homogeneous precipitation method (Duan et al., 2006). The morphology of the synthesized ZnO NPs was characterized by the SEM, which displayed an evenly dispersed phase of ZnO NPs with diameters of 50–200 nm (Fig. S1). 0.5 M stock solution was prepared by dissolving a required amount of ZnO NPs in distilled water (Kasemets et al., 2009). Then the prepared ZnO NPs solution was diluted to 10, 50 and 100 mg/L for shock loading in present study (Hou et al., 2013; Quan et al., 2015; Xu et al., 2016).

### 2.2. Set-up and operation of the granular SBR

The aerobic granular sludge used for the ZnO NPs exposure experiments was from the previous granular SBR with sodium acetate as the sole carbon source (He et al., 2016b). The granules were with an average diameter of  $1.5 \pm 0.5$  mm and an initial sludge

volume index at 5 min (SVI<sub>5</sub>) of  $22.58 \pm 0.69$  mL/g. The concentration of the mixed liquor suspended solids (MLSS) in each reactor was  $4.4 \pm 0.5$  g/L.

Plexiglas reactors were 100 mm in inner diameter and 500 mm in height, giving an effective volume of 3.6 L and a height/diameter (H/D) of 5 (Fig. 1). 1.8 L of synthetic wastewater was pumped into the reactor at the beginning of every cycle. The air was introduced by a fine-bubble aerator from the bottom of the reactor with a constant airflow rate of 2.5 L/min, and the dissolved oxygen (DO) concentration was controlled at about 5.0 mg/L during the aerobic phase. A stirring speed of 250 rpm was set throughout the anaerobic, aerobic and anoxic phases by a mechanical stirrer. The water temperature of the reactor was not controlled ( $19 \pm 2$  °C).

The reactor was operated on a 6-h-cycle, consisting of 2 min of feeding, 120 min of anaerobic phase, 90 min of aerobic phase, 144 min of anoxic phase, 2 min of settling time and 2 min of effluent discharge periods. The sodium acetate-based synthetic wastewater was composed of (per liter): 150 mg chemical oxygen demand (COD), 18 mg ammonia nitrogen ( $NH_4^+-N$ ), 3.5 mg total phosphorus (TP), 10 mg  $Ca^{2+}$ , 10 mg  $Mg^{2+}$ , and 1 mL trace solution (He et al., 2016c).

### 2.3. Evaluation of microbial community of AGS under ZnO NPs exposure

The effects of ZnO NPs on the microbial community of AGS were determined based on the oxygen uptake rate (OUR), ammonia oxidizing rate, phosphorus release rate, aerobic phosphorus uptake rate and phosphorus uptake rate. The measurement of OUR was conducted according to our previous research (He et al., 2016c).

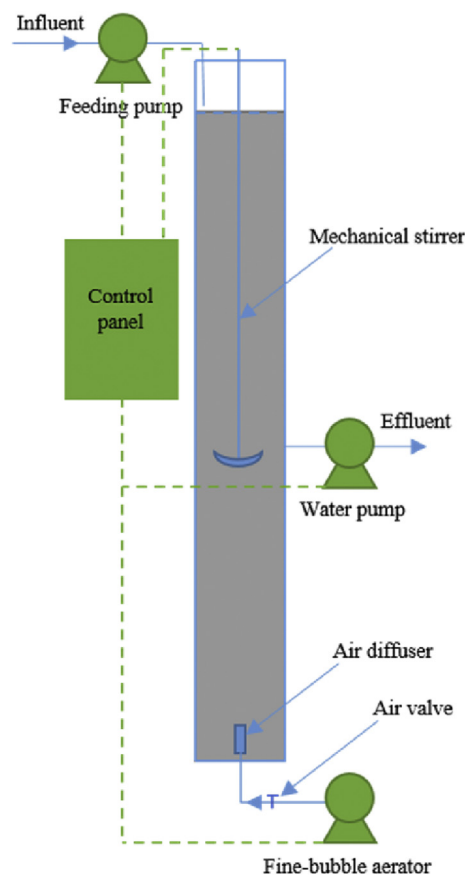


Fig. 1. Schematic diagram of the sequencing batch reactor.

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