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Understanding the performance of microbial community induced by ZnO nanoparticles in enhanced biological phosphorus removal system and its recoverability

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HIGHLIGHTS

- ZnO NPs (above 2 mg/L) could seriously deteriorate the performance of EBPR system.
- The microbial community structure in the EBPR system was largely changed by ZnO NPs.
- *Competibacter* was more sensitive than *Accumulibacter* after exposure to 2 mg/L ZnO NPs.
- *Accumulibacter* had stronger recoverability compared with *Competibacter*.

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ABSTRACT

In this study, the impacts of ZnO Nanoparticles (NPs) on the microbial community in enhanced biological phosphorus removal (EBPR) system and its recoverability were investigated. High-throughput sequencing was applied to study the microbial community shift. Results show that the species richness in the EBPR system was reduced under the condition of ZnO NPs with high concentration (above 6 mg/L). Evolution analysis suggests that higher concentration ZnO NPs induced more microbial community shift. According to the analysis on genus level, *Competibacter* was more impressionable than *Accumulibacter* after exposure to 2 mg/L ZnO NPs. Nonetheless, this phenomenon could not be found as the concentration of ZnO NPs got higher (above 6 mg/L). *Accumulibacter* could reach to the initial level after recover for 20 days, whereas *Competibacter* could not recover even when the concentration of ZnO NPs was only 2 mg/L. Interestingly, although the phosphorus removal (P-removal) process was re-achieved, the microbial community in reactors was irreversible.

1. Introduction

Phosphorus is the main element to induce eutrophication, which has become an important water quality problem worldwide (Zheng et al., 2014). Enhanced biological phosphorus removal (EBPR) system has been widely accepted as the most economic and sustainable process for removing phosphorus from wastewater to control eutrophication problems (Lv et al., 2013). The performance of an EBPR system operating in an anaerobic/aerobic (or anoxic) configuration depends on the enrichment of phosphorus accumulating organisms (PAOs) (Ye et al., 2010; Zheng et al., 2013a). Therefore, the high enrichment of PAOs plays an important role in achieving a high efficiency of biological phosphorus removal. Under anaerobic conditions, PAOs take up carbon source such as volatile fatty acids (VFAs) and store them in the form of polyhydroxyalkanoates (PHAs). The energy for this process is mainly from hydrolysis of the intracellular stored polyphosphates (poly-P), resulting in ortho-phosphate release into solution. Under aerobic or anoxic conditions, PAOs are able to uptake excess phosphorus and store them as intracellular poly-P by using stored PHAs as the energy source. The net P removal can be achieved through wasting activated sludge which is enriched in poly-P. Being regarded as another important microbial community in an EBPR system, glycogen accumulating organisms (GAOs) can compete with PAOs for the carbon source in anaerobic stage, which should be studied as well.

Recently, inhibitory factors on EBPR system, such as antibiotics, metal ion, free ammonia, nitrite and high concentration chemical oxygen demand (COD), have been widely reported in many researches (Hu et al., 2016; Motlagh et al., 2015; Fang et al., 2015; Zheng et al., 2013a,b; Yu et al., 2014). Some researches

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indicted that free ammonia (FA) (above 17.76 mg N/L), nitrite (above 10 mg/L) and COD (above 600 mg/L) could seriously destroy the process of P-removal within a long-term (Zheng et al., 2013a,b; Yu et al., 2014). Meanwhile, metal ion such as chromium (Cr) (above 5 mg/L) and silver (Ag) (above 5 mg/L) could also destroy the process of P-removal within a short-term period (Fang et al., 2015; Chen et al., 2012). However, many of them payed attention to the microbial community shift in EBPR induced by inhibitory factors (Zheng et al., 2013a,b). Up to now, the influences of some emerging contaminants, especially nanoparticles (NPs), on EBPR system are few reported. Although Chen et al. (2012) investigated the effect of Ag NPs on EBPR system, and concluded that the phosphorus removal efficiency was maintained at 99.0% under the effect of 5 mg/L Ag NPs after a short-term experiment (one cycle). It is insufficient to understand the effects of NPs comprehensively, especially on the aspect of microbial community shift induced by NPs.

Nanotechnology has been widely applied in various areas, such as catalysts, cosmetics, electronics and wastewater treatment (Gottschalk et al., 2009; Oberdörster et al., 2005; Qu et al., 2013). The extensive applications of NPs would inevitably bring large amounts of NPs into the environment via effluent or surface runoff. Moreover, wastewater treatment plants (WWTPs) as the final receptors of most NPs, will be largely shocked by NPs due to its large specific surface area and unique catalytic properties (Li et al., 2014). It is more difficult to biodegrade NPs than traditional organic pollutants through activated sludge (Bakheet et al., 2013). Consequently, it is necessary to fully understand the effect of NPs on activated sludge.

ZnO NPs, due to its unique electronic and optical properties, have been widely used in semiconductor, sensors and photonic crystals (Hou et al., 2014). Compared with TiO_2 and SiO_2 NPs, ZnO NPs have the most adverse effects (Yu et al., 2015). A recent study confirmed that ZnO NPs in waste water treatment plants (WWTPs) were hazardous to the activated sludge microbial community (Wang et al., 2016). Earlier studies have also found that denitrifying bacteria and PAOs could be more seriously inhibited by high concentration (above 10 mg/L) ZnO NPs than nitrobacterium (Hou et al., 2013). Low concentration (1 mg/L) ZnO NPs showed significant effect on neither phosphorus nor nitrogen removal in a short-term experiment, but the adverse effect was measurable after a long-term exposure (Puay et al., 2014). While Zheng et al. (2011) suggested that high concentration (10 and 50 mg/L) ZnO NPs could destroy the process of biological nitrogen and phosphorus removal within a short-term in activated sludge system. However, whether the presence of ZnO NPs can induce microbial community shift, especially PAOs and GAOs shift in EBPR system, has not been well investigated so far. Furthermore, no research has focused on the recoverability of PAOs and GAOs after the effect of ZnO NPs in EBPR system.

The main objective of this study is to investigate the long-term effect of ZnO NPs on microbial community in EBPR system and its recoverability. In order to gain a detailed understanding of the influence of ZnO NPs on microbial community in EBPR system, high-throughput sequencing was adopted to reveal the changes in the phylum, class and genus levels of microbial community in the EBPR system respectively.

2. Materials and methods

2.1. ZnO nanoparticles and sludge

ZnO NPs (15–30 nm, uncoated) were purchased from Hangzhou Wanjing New Material Ltd, China. Stock dispersions of ZnO NPs were prepared in deionized water and sonicated using a 130-Watt ultrasonic processor (Cole-Parmer Instruments, Vernon Hills, IL, USA) at 100% amplitude for 30 min (Sun et al., 2014b).

Activated sludge was obtained from the Hangzhou Qige Municipal wastewater treatment plant, China. Anaerobic-anoxic-aerobic process was used in the domestic wastewater treatment plant. The main parameters of concentrated sludge were as following: mixed liquor suspended solids (MLSS) 7.8 ± 0.5 g/L, the ratio of mixed liquor volatile suspended solid (MLVSS) to MLSS was 57 ± 6%, pH was 6.8–7.5, and SRT was 25–30d.

2.2. Synthetic wastewater

Synthetic wastewater was prepared for experiment (containing 30 mg P/L phosphate, and 600 mg COD/L of acetate and propionate in the ratio of 1/3 as the mixed carbon source). The synthetic wastewater consisted of (per liter water): 0.256 g CH3COONa, 0.4 mL CH_3CH_2COOH, 0.2293 g NH_4Cl, 0.0875 g KH_2PO_4, 0.147 g $K_2HPO_4{\cdot}3H_2O$, 0.09 g $MgSO_4$, 0.0222 g $CaCl_2$, 0.0015 g peptone, 0.0015 g yeast extract powder, 0.0072 g allylthiourea (ATU) and 0.6 mL trace elements solution. 1.8 mg/L Allylthiourea (ATU) was added to inhibit nitrifiers. Moreover, the trace elements solution prepared according to Smolders et al. (1994).

2.3. Batch experiments

One 10 L lab-scale anaerobic-aerobic sequencing batch reactor (SBR) fed with 3.3 L synthetic wastewater was used to enrich PAOs. The batch experiments were operated at 21 ± 1 °C and for three cycles each day. Each cycle (8 h) consisted of following steps in order, 5 min for feeding synthetic wastewater, 120 min for anaerobic phase, 180 min for aerobic phase, 20 min for sedimentation, 5 min for extracting 3.3 L supernatant and 150 min for idling. The hydraulic retention time (HRT) was 24 h. The synthetic wastewater was constantly mixed with a magnetic stirrer except for the settling, decanting, and idling steps. The agitation speed during the operation was controlled at around 180 rpm. The highest dissolved oxygen (DO) level during the aerobic phase was at 6–7 mg L^{-1}. The pH was adjusted to 7.0–7.5 with 0.5 M NaOH and 0.5 M HCl during the operation. Sludge was wasted to keep the solids retention time (SRT) at approximately 7–9 days. After three months, stable phosphorous removal efficiencies were observed in the parent SBR.

Batch experiments were carried out with the sludge of each reactor to examine the impact of ZnO NPs on P-removal process and microbial community structure. Four parallel SBR reactors (termed as R1, R2, R3 and R4) with the working volume of 10 L were used in this study. R1 was used as control, where no ZnO NPs as added. ZnO NPs were added to keep the influent ZnO NPs concentration in the R2, R3 and R4 at 2 mg/L, 6 mg/L and 10 mg/L respectively. In the recover stage (from Day 24 to Day 43), no ZnO NPs was added in each reactor.

2.4. DNA extraction and high-throughput sequencing

Samples were collected from the activated sludge in the SBR before ZnO NPs were added (R1), 14 (Day 14) and 23 days (Day 23) after the ZnO NPs were fed and after recover for 20 days (Day 43). Each DNA sample was extracted by using the 3S DNA Isolation Kit for Environmental Samples (Shanghai Biocolor bioscience & technology, China) in accordance with manufacturer's instructions. PCR was performed using the primers 515F (5′-GTGCCAGC MGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). PCR was performed in TransStart Fastpfu DNA Polymerase with a 20 μL reaction containing 10 ng of genomic DNA, 4 μL of 5 × reaction buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of Forward Primer (5 μM), 0.8 μL of Reverse Primer (5 μM) and 0.4 μL of FastPfu Polymerase. The amplification conditions consisted of an initial denaturation