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Effects of CeO_2 nanoparticles on bacterial community and molecular ecological network in activated sludge system☆

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ABSTRACT

The increasing use of cerium oxide nanoparticles (CeO_2 NPs) has caused concerns regarding their potential environmental risks. However, their effects on bacterial communities and network interactions in activated sludge process are still unclear. In this study, we carried out long-term exposure experiments (210 d) to investigate the influence of CeO_2 NPs on wastewater treatment performance, bacterial community structure and network interactions in activated sludge systems. The results showed that long-term exposure to 1 mg/L CeO_2 NPs induced the deterioration of denitrifying process, which was consistent with the inhibition of enzyme activities of nitrite oxidoreductase and nitrate reductase under CeO_2 NPs. CeO_2 NPs decreased the bacterial diversity and altered the overall bacterial community structure in activated sludge. Some dominant denitrifying bacteria, such as *Flexibacter* and *Acinetobacter* decreased significantly. Molecular ecological network analysis showed that CeO_2 NPs decreased the network complexity of bacterial community, and probably promoted the competition in bacterial communities of activated sludge. These changes of denitrifying bacteria and the bacterial network may be relevant to the deterioration of denitrifying process. This study provides insights into how the bacteria community and their molecular ecological network respond to CeO_2 NPs in activated sludge systems.

1. Introduction

Cerium oxide nanoparticles (CeO_2 NPs) are widely used in various commercial applications such as catalysis and catalyst support, superconducting materials, fuels, ceramics, corrosion coatings and personal care products (Wang et al., 2016a). The large production and utilization of CeO_2 NPs inevitably cause their discharge into the wastewater collection systems during their manufacturing, transport, utilization and disposal process, and then they finally enter biological wastewater treatment plants (WWTPs). Previous studies have shown that CeO_2 NPs were highly toxic to some microorganisms, such as *Nitrosomonas europaea* (Fang et al., 2010), *Pseudokirchneriella subcapitata* (Cerrillo et al., 2016), and *Chlamydomonas reinhardtii* (Kosak Nee Rohder et al., 2018). The toxic properties of CeO_2 NPs suggest their potential adverse effect on the performance of WWTPs.

Some preliminary studies have evaluated the effects of CeO_2 NPs on activated sludge system. In a sequencing batch biofilm reactor (SBBR), Hou et al. (2015) found that short term exposure to 1 mg/L CeO_2 NPs had no significant effect on total nitrogen (TN) removal, while 10 and 50 mg/L CeO_2 NPs significantly reduced TN removal efficiency. Wang et al. (2016a) showed that high concentrations of CeO_2 NPs (60 mg/L) could result in the biotoxicity to activated sludge, but did not have obvious effect on the microbial diversity of activated sludge. However, these studies mainly focus on the short-term effects of CeO_2 NPs on the treatment performance, and how microbial communities respond to long-term exposure of CeO_2 NPs in activated sludge system is worth studying.

Activated sludge system is a complex ecosystem, in which various functional bacteria live together to accomplish system level functions (e.g., removal of oxygen-depleting organics and nutrients) through various types of interactions (Gou et al., 2016; Pang et al., 2016). These interactions could be positive, negative or neutral (no impact on the species involved). Positive relationships mainly result from mutualism or commensalism, while negative relationships are likely due to competition, ameensalism, predation

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and so on (Faust and Raes, 2012). It is expected that the entering of NPs could altered the microbial network interactions in activated sludge system. However, the effects of CeO_2 NPs on the network interactions of activated sludge bacterial populations still remain unclear.

The major objectives of this study were to: (i) examine the long-term effects of CeO_2 NPs on the bacterial community structure and diversity and (ii) elucidate how network interactions of bacterial community respond to CeO_2 NPs in activated sludge system. For these purposes, three lab-scale sequencing batch reactors (SBRs) subjected to 1 mg/L CeO_2 NPs and three control SBRs were carried out over an extended period (210 d). MiSeq sequencing and a random matrix theory (RMT)-based network method were used to assess the responses of bacterial communities and their network interactions to CeO_2 NPs.

2. Materials and methods

2.1. Preparation of CeO_2 NPs suspension

Commercially produced CeO_2 NPs was purchased from Nano-Amor (New Mexico, America). The size and morphology of the CeO_2 NPs was characterized by transmission electron microscope (TEM) (Fig. S1). The NPs were nearly spherical and the average particle size calculated was about 30 nm CeO_2 NPs were confirmed to be pure by X-ray diffraction (XRD) (Ultima IV Diffractometer, Rigaku, Japan) (Fig. S2). Stock suspensions of CeO_2 NPs were made by adding 100 mg CeO_2 NPs to 1.0 L Milli-Q (Molsheim, France) water, followed by 1 h of ultrasonication (25 °C, 120 W, 40 kHz). The average hydrodynamic diameter, particle size peak, and zeta potential in two different liquid media (deionized water and the influent wastewater of SBR) were determined by a Zetasizer Nano ZS (Malvern Instruments, Uk). In this study, 1 mg/Lwas chosen as the environmentally relevant concentration of CeO_2 NPs in WWTPs (Hou et al., 2015; Limbach et al., 2008).

2.2. Activated sludge bioreactors

Three lab-scale SBRs subjected to 1 mg/L CeO_2 NPs (CeO_2 SBRs) and three controls were operated in parallel. Each SBR with 11 cm inner diameter and 50 cm height was made of plexiglass cylinder. Fig. S3 presents the schematic diagram of the SBR. At the beginning of reactor operation, each reactor was inoculated 2000 mL mixed liquor taken from the aeration basin of a local municipal WWTP (Beijing, China). All the reactors were run for about 30 days to achieve stable performance. The operational process of each circle consisted of anaerobic stage (90 min), aerobic stage (140 min), anoxic stage (60 min), settling stage (90 min), discharge stage (10 min) and idle stage (90 min). In each cycle, 2 L of synthetic wastewater were pumped into the reactor in the first 15 min of the anaerobic stage. The synthetic wastewater was used containing per liter (mg): Glucose (515.63), NH_4Cl (114.64), KH_2PO_4 (21.93), $MgSO_4 \cdot 7H_2O$ (90), $CaCl_2 \cdot 2H_2O$ (14), and 0.3 mL nutrient solution. The nutrient solution contained (g/L): $FeCl_3 \cdot 6H_2O$ (1.5), H_3BO_3 (0.15), $CuSO_4 \cdot 5H_2O$ (0.03), KI (0.18), $MnCl_2 \cdot 4H_2O$ (0.12), $Na_2MoO_4 \cdot 2H_2O$ (0.06), $ZnSO_4 \cdot 7H_2O$ (0.12), $CoCl_2 \cdot 6H_2O$ (0.15), and EDTA (10). The influent concentrations of chemical oxygen demand (COD), ammonia nitrogen (NH_4^+-N) and total phosphorus (TP) were approximately 500, 30, and 5 mg/L, respectively. The CeO_2 NPs were added into the influent after the SBR operation reached steady state. The solids retention time (SRT) and hydraulic retention time (HRT) were 20 d and 16 h, respectively. The mixed liquid suspended solids (MLSS) were around 3500 mg/L.

2.3. Analytical methods

For wastewater treatment performance analysis, wastewater samples were collected every 2–3 days in the first 90 days, and every 3–5 days in the last 120 days. Totally, 60 samples for each SBR were taken. COD, NH_4^+-N, nitrite (NO_2^--N), nitrate (NO_3^--N), TP, MLSS and mixed liquor volatile suspended solids (MLVSS) were determined according to the Standard Methods (Albertsen et al., 2012). After acidification with HNO_3 to $pH \leq 2$, CeO_2 in liquid samples was measured by inductively coupled plasma-optical emission spectroscopy ICP-OEM7700 (Agilent Technologies, USA), and the methods were detailed in the Supporting Information (SI). On the last day of the operation period, activated sludge samples were collected for enzyme activity analysis. The activities of ammonia monooxygenase (AMO), nitrite oxidoreductase (NOR), exopolyphosphatase (PPX), polyphosphate kinase (PPK), nitrate reductase (NAR) and nitrite reductase (NIR) were detailed in SI (Zheng et al., 2011a).

2.4. DNA extraction and sequencing

Activated sludge samples were weekly collected from each SBR at the aerobic stage during the final four weeks of the study period. Totally, 24 activated sludge samples were collected. DNA was extracted by using a FastDNA® SPIN Kit for Soil Kit (MP Biotechnology, USA) according to the manufacturer's protocol. For Illumina sequencing, the primers 515F (5′-GTG CCAG CMGC CGCG GTAA-3′) and 806R (5′-GGAC TACH VGGG TWTC TAAT-3′) were used to amplify the V4 region of the 16S rRNA gene. Polymerase chain reaction (PCR) was conducted according to our previous literature (Hai et al., 2014). 16S rRNA sequencing was performed on Illumina MiSeq platform at a commercial company (Majorbio, China). Quality filtering of the raw reads were performed according to our previous literature (Wang et al., 2012).

2.5. Statistical analysis

Shannon-Wiener was used to evaluate the bacterial diversity. Principal coordinate's analysis (PCoA) was conducted to examine the overall variation among activated sludge bacterial communities. All the statistical analyses were conducted by using the VEGAN package in R (v.3.4.1; http://www.r-project.org/). Some assays were performed in triplicate or more and an analysis of variance (ANOVA) was carried out to examine the significance of results.

2.6. Network construction

To study the effect of CeO_2 NPs on the bacterial interactions, two Molecular Ecology networks (MENs) were constructed for CeO_2 SBRs and controls, respectively, using the online MENA pipeline by following these steps (IEG, the University of Okalahama, America) (Deng et al., 2016; Tu et al., 2015). First, the relative abundance of individual operational taxonomic unit (OTU) was used to construct a Pearson correlation matrix. Second, the Pearson corelation matrix was converted to a similarity matrix which measures the degree of concordance between the abundance profiles of OTUs across different samples (Horvath and Dong, 2008). Third, an adjacency matrix showing the connection strength between each pair of nodes was constructed based on the similarity matrix by applying an appropriate threshold, *st* (Luo et al., 2006, 2007). Fourth, fast greedy modularity optimization was used to detect the modules (Clauset et al., 2004; Zhou et al., 2010). For statistically network comparing, Maslov-Sneppen procedure was performed to assess the statistical significance of network indices (Liang et al., 2016;