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Long term effects of cerium dioxide nanoparticles on the nitrogen removal, micro-environment and community dynamics of a sequencing batch biofilm reactor

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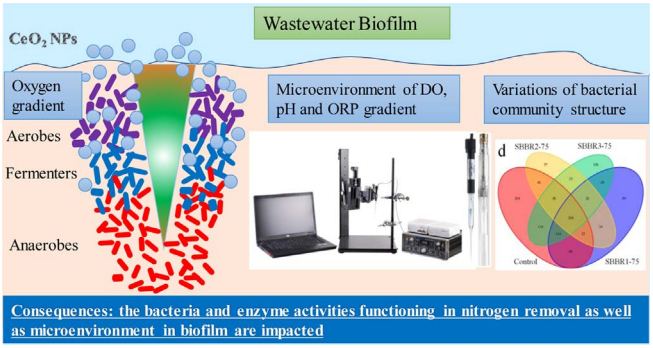
GRAPHICAL ABSTRACT



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ABSTRACT

The influences of cerium dioxide nanoparticles (CeO_2 NPs) on nitrogen removal in biofilm were investigated. Prolonged exposure (75 d) to 0.1 mg/L CeO_2 NPs caused no inhibitory effects on nitrogen removal, while continuous addition of 10 mg/L CeO_2 NPs decreased the treatment efficiency to 53%. With the progressive concentration of CeO_2 NPs addition, the removal efficiency could nearly stabilize at 67% even with the continues spike of 10 mg/L. The micro-profiles of dissolved oxygen, pH, and oxidation reduction potential suggested the developed protection mechanisms of microbes to progressive CeO_2 NPs exposure led to the less influence of microenvironment, denitrification bacteria and enzyme activity than those with continuous ones. Furthermore, high throughput sequencing illustrated the drastic shifted communities with gradual CeO_2 NPs spiking was responsible for the adaption and protective mechanisms. The present study demonstrated the acclimated microbial community was able to survive CeO_2 NPs addition more readily than those non-acclimated.

1. Introduction

Due to the novel physicochemical properties, cerium dioxide nanoparticles (CeO_2 NPs) have been implemented in diverse applications related to catalysts, textiles, pharmaceutics and biomedical products (Abreu and Morais, 2010). The increasing production and utilization of CeO_2 NPs inevitably resulted in their release into the environment with the life cycle of manufactured goods, which have been estimated by the

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means of empirical studies and models (Limbach et al., 2008). While recent research documented that once CeO_2 NPs reached the environment, they would pose potential threats to both the human health and ecosystem, owing to their interactions with microorganisms (Wang et al., 2012). As NPs enter into wastewater streams and eventually end up at wastewater treatment plants (WWTPs), García et al. (2012) reported that CeO_2 NPs influenced the respiration rate of ammonia bacteria, anaerobic bacteria and heterotrophic bacteria from a municipal WWTP. Besides, the acute deterioration in the performance of biological nitrogen removal and microbial enzymatic activity caused by CeO_2 NPs in sequencing batch biofilm reactor (SBBR) have been demonstrated by Hou et al. (2015). However, little information is available to estimate the long term toxicological effects of CeO_2 NPs on the nitrogen removal performance as well as the related mechanisms in SBBR system.

SBBR is a well established technology for wastewater treatment where bacteria grow to form biofilm matrix with a three-dimensional structure on the carriers (Stewart and Franklin, 2008). Nitrogen removal has been successfully achieved in SBBRs, since the carriers can provide a suitable surface for nitrifying bacteria (including ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB)) and denitrifying bacteria (DNB) with slow growing rate. The simultaneous nitrification and denitrification (SND) can also be realized in the biofilm treatment processes to complete the nitrogen removal process in one structure (Yang et al., 2014), for the dissolved oxygen (DO) concentration gradients result from diffusional limitations (Puznava et al., 2001). First, oxidation of ammonia to nitrate is accomplished by AOB and NOB under aerobic and anoxic conditions. Then, anoxic microorganisms mainly distributed in the center or inner part of the biofilm that allows denitrifiers to produce nitrogen in the traditional way. While current knowledge about the toxicity effects of CeO_2 NPs on microbial community is based on the pure culture (Yu et al., 2015) or activated sludge (Wang et al., 2016), information about the long-term toxicity effects of CeO_2 NPs on the specific microbial community including AOB, NOB and DNB is still limited.

In addition to the microbial community, the variations of microenvironment in biofilm are also important factors influencing the performance of SBBR system (Wen et al., 2016). Pellicer-Nàcher et al. (2013) studied the oxygen profiles in biofilm and further analyzed the relationship between the aerobic zone and nitrogen removal in membrane-aerated nitrating biofilm reactors. The micro-gradients of DO throughout biofilms were also related to the profiles of oxidation reduction potential (ORP) (Wen et al., 2016), which corresponded to the metabolic activity of the biofilm (Yu and Bishop, 1998). Furthermore, studies have declared that both DO and pH are the most important factors influencing denitrifying performance of biofilms (Clauwaert et al., 2009; Watanabe et al., 2001). In our previous study, the spatial distribution of O_2 in biofilm were determined to be increased after exposure to 50 mg/L ZnO NPs (Hou et al., 2014). Nevertheless, the micro-environment pH in biofilm was measured to be decreased after exposure to 50 mg/L CuO NPs and the decreased pH led to the inhibited enzymes activity (Hou et al., 2016). Therefore, better understanding the long-term exposure effects of CeO_2 NPs on the variations of micro-environment (DO, pH, ORP) of biofilm is necessary to evaluate the activities of microbial community and functional enzymes as well as the biochemical reactions involved in the nitrogen removal. In general, gradual exposure of microbes to stress may strengthen them through the adaption (Alito and Gunsch, 2014), while the cumulative effect may weaken them. In this point of view, responsive mechanisms and susceptibility of bacteria to CeO_2 NPs progressive exposure may be distinct from that of a single concentration exposure and thus were investigated in this study.

The present study was conducted to investigate the long-term effects of CeO_2 NPs on wastewater biofilm, mainly focusing on the following aspects: (1) removal efficiency of total nitrogen (TN) during the long-term exposure process; (2) variations of AOB, NOB and DNB abundance as well as the key microbial enzymatic activities; (3) long term effects of CeO_2 NPs on the micro-environment inside biofilm; (4) responses of microbial community to CeO_2 NPs exposure in terms of richness, diversity and composition.

2. Materials and methods

2.1. Biofilm culturing and CeO_2 NPs suspension

SBBR with an operating volume of 3 L and treating 2.5 L of wastewater per cycle was employed in this study. During the culturing period, each cycle comprised a 2 min filling phase, 5 h aeration recirculation, 3 h anaerobic reaction and 5 min draining, as previously described in our study (Xu et al., 2016). After the combined packing acting as carriers for microorganism attachment was suspended in the reactors, activated sludge taken from secondary sedimentation tank was inoculated in each reactor with concentration of 3.5 g/L biomass. 2.5 L of synthetic wastewater was then added into the reactor with the predetermined concentrations of chemical oxygen demand (COD) of 250 mg/L, TN 27 mg/L and total phosphorus (TP) 6.25 mg/L. Glucose served as a carbon source and the remaining components were $C_6H_{12}O_6$ (1270 μM), NH_4Cl (1221 μM), $NaNO_3$ (735 μM), $CaCl_2$ (170 μM), $Na_2HPO_4{\cdot}12H_2O$ (400 μM), $MgCl_2{\cdot}6H_2O$ (70 μM), $FeSO_4{\cdot}7H_2O$ (31 μM), $ZnSO_4{\cdot}7H_2O$ (15 μM), $(NH_4)_6Mo_7O_{24}$ (0.12 μM), $MnSO_4{\cdot}H_2O$ (3.38 μM), $CuSO_4{\cdot}5H_2O$ (1.15 μM).

Commercially accessible CeO_2 NPs (purity: > 99%) powders were obtained from Sigma-Aldrich (St. Louis, MO) and the particle diameter is less than 50 nm, with a specific surface area of 30 m^2/g. A visual inspection of CeO_2 NPs (Fig. A.1a, Supplementary Material) was performed using a scanning electron microscopy (SEM, Hitachi S-4800). X ray diffraction (XRD, SmartLab, Rigaku) analysis of CeO_2 NPs was conducted to observe the properties of phase structure and exhibited in Fig. A.1b. The stock suspension of CeO_2 NPs was obtained by mixing 100 mg CeO_2 NPs with 1 L Milli-Q water (pH 7.0) and ultrasonicated for 1 h to break the aggregates at a power output of 250 W and 40 kHz at indoor temperature (20 °C) prior to the exposure experiments (Hou et al., 2015).

2.2. Establishment of the long-term exposure experiment

Exposure experiments were carried out when a series of SBBRs reached a stable TP, TN and COD removal efficiency after culturing for months. In the present study, 0.1 mg/L CeO_2 NPs was investigated as the environmentally relevant concentration in WWTPs according to Limbach et al. (2008) and marked as SBBR 1. However, considering the increasing production and utilization of CeO_2 NPs (more than 10000 t $year^{-1}$) (Lazareva and Keller, 2014), 10 mg/L was also detected and denoted as SBBR 3. Particularly, in SBBR 2, CeO_2 NPs concentration was increased from 0.1 to 10 mg/L including 0.1, 1, 5 and 10 mg/L at Stage I (Day 0 to 5), Stage II (Day 6 to 15), Stage III (Day 16 to 30) and Stage IV (Day 31 to 75), respectively. CeO_2 NPs treatment experiments lasted for 75 days and the reactor with no CeO_2 NPs was used as Control. The different concentrations of CeO_2 NPs in the influent were achieved by mixing the CeO_2 NPs stock suspension with the synthetic wastewater and fed to the SBBR in each cycle.

2.3. Microelectrode measurements

Microelectrode is one of the promising and critical tools to quantify local chemistry in biofilms, including in situ sensing, minimal invasive and no influencing on biofilm structure (Hou et al., 2014). The microelectrode automation system (PA2000, unisense, Denmark) consisted of three parts: a computer program, a data acquisition system and a motion control system. The tip diameters of the combined microelectrodes are 100 μm. Before each application, the microelectrodes are polarized for at least 12 h and calibrated in accordance with the