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Short Communication

Effects of cerium oxide nanoparticles on the species and distribution of phosphorus in enhanced phosphorus removal sequencing batch biofilm reactor

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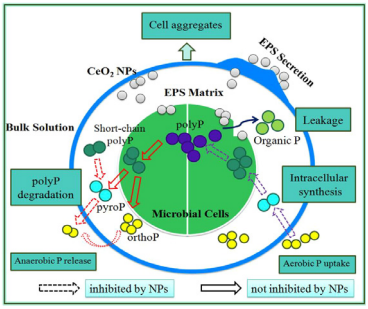
HIGHLIGHTS

- 20 mg/L CeO_2 NPs posed adverse effects on the process of P removal in biofilm.
- The transformation of P in EPS was influenced during anaerobic period.
- The formation of polyP in cells and EPS declined after aerobic exposure.
- The characterizations and roles of EPS in P removal were impacted.

GRAPHICAL ABSTRACT



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ABSTRACT

The short term (8 h) influences of cerium oxide nanoparticles (CeO_2 NPs) on the process of phosphorus removal in biofilm were investigated. At concentration of 0.1 mg/L, CeO_2 NPs posed no impacts on total phosphorus (TP) removal. While at 20 mg/L, TP removal efficiency reduced from 85.16% to 59.62%. Results of P distribution analysis and ^{31}P nuclear magnetic resonance spectroscopy implied that the anaerobic degradation of polyphosphate (polyP) and the release of orthophosphate in extracellular polymeric substances (EPS) were inhibited. After aerobic exposure, the average chain length of polyP in microbial cells and EPS was shorter than control, and monoester and diester phosphates in cells were observed to release into EPS. Moreover, the EPS production and its contribution to P removal increased, while the capacity of EPS in P storage declined. X-ray diffraction analysis and saturation index calculation revealed that the formation of inorganic P precipitation in biofilm was inhibited.

1. Introduction

Cerium dioxide nanoparticles (CeO_2 NPs) have been implemented in diverse applications related to catalysts, textiles, pharmaceutics and biomedical products (Abreu and Morais, 2010; Patil et al., 2002). The increasing production and utilization of CeO_2 NPs inevitably results in their release into wastewater treatment plants. The deterioration in the performance of biological nitrogen removal caused by CeO_2 NPs in sequencing batch biofilm reactor (SBBR) have been demonstrated by Hou et al. (2015). While the influence of CeO_2 NPs on the enhanced biological P removal (EBPR) in SBBR have not been fully understood.

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As a promising biological wastewater treatment system, SBBR was designed to feed and drain periodically under alternating anaerobic and aerobic conditions for phosphorus removal (Chiou and Yang, 2008), which was mainly attributed to a group of selectively enriched bacteria, phosphorus accumulating organisms (PAOs). PAOs in SBBR achieve polyphosphate (polyP) accumulation through the release of orthophosphate (orthoP) in anaerobic phase, and then "luxury uptake" of orthoP in the aerobic phase, transforming orthoP to polyP. In this way, net P removal is realized by discarding the P-rich excess biomass (Oehmen et al., 2007).

Compared to the flocculent and granular sludge, the special 3-D structure as well as the quantities of extracellular polymeric substances (EPS) are expected to substantially enhance the ability of biofilm in absorbing and removing P more effectively (Stewart and Franklin, 2008). Moreover, a significant level of P accumulation in EPS was observed (Huang et al., 2015; Zhang et al., 2013), revealing the vital role of EPS in the EBPR process. Nevertheless, information about the transformation of P among bulk liquid, EPS and cell clusters as well as the contribution of EPS and biofilm residue to P removal after exposure to CeO_2 NPs in SBBR is very limited. In addition to the dynamic migration of P in biofilm, identification of P species in EPS and cells is helpful to address the characteristics of P in biofilm after exposure to NPs and thus the potential toxicity mechanisms.

The aim of this study is to investigate the effects of CeO_2 NPs on the fractionation and distribution of P in SBBR systems under anaerobic and aerobic conditions. 31P nuclear magnetic resonance (NMR) spectroscopy was employed to visualize the inorganic and organic P species in both EPS and pellets. X-ray diffraction (XRD) analysis was used to further determine the inorganic P forms of the precipitates in the treated and control biofilm.

2. Materials and methods

2.1. CeO_2 NPs characteristics

Commercially produced CeO_2 NPs (purity: >99%) powders with the particle diameter of less than 50 nm were obtained from Sigma-Aldrich (St. Louis, MO). A visual inspection of CeO_2 NPs (Fig. A.1, Supplementary material) was conducted using a scanning electron microscopy (SEM, Hitachi S-4800). 100 mg of CeO_2 NPs were added to 1 L Milli-Q water (pH 7.0), and the resulting stock suspension (100 mg/L) was ultrasonicated with probe for 1 h (20 °C, 250 W, 40 kHz) (Hou et al., 2015) prior to the exposure experiments. Analysis of particle-size distribution and zeta potential of CeO_2 NPs in the process of exposure were examined with a Malvern Zetasizer Nano ZSP (Malvern Instruments, UK). The characterizations of CeO_2 NPs were listed in Table A.1 in the Supplementary Material.

2.2. Biofilm matrix and exposure experiments

A SBBR with an operating volume of 3 L and treating 2.5 L of wastewater per cycle was employed in this study to cultivate biofilm. After the combined packing acting as carriers for microorganism attachment was suspended in the reactor, activated sludge taken from secondary sedimentation tank was inoculated in each reactor with the concentration of 3.5 g/L biomass. The reactor operation parameters and the components of synthetic wastewater are same with a previous work (Xu et al., 2016).

For the exposure experiments, 0, 0.1 and 20 mg/L CeO_2 NPs were examined when the total phosphorus (TP) removal efficiency reached a stable state after 70 days' operation. Then the corresponding bioreactor was marked as SBBR 0, SBBR 1 and SBBR 2. The detailed procedures are provided in Xu et al. (2016). In the cycle, two pieces of carriers were taken at 0, 3 and 8 h and then the biofilm was scraped and resuspended in 100 mL synthetic wastewater. Two 25 mL suspension were used for detection of TP and inorganic P in biofilm and a 50 mL for EPS extraction.

2.3. Extraction and species analysis of P in EPS and cells

Cation-exchange resin (CER) extraction method was used for EPS extraction from the biofilm (Fig. A.2a). The cold perchloric acid (PCA) fractionation combined with NaOH extraction approach was applied to fractionate P in microbial cells and the details were provided in Fig. A.2b. The extracts were lyophilized at −50 °C for more than 48 h and stored at −20 °C until ^{31}P NMR analysis. Besides, 0.1 g of sodium hexametaphosphate (chosen as polyP model compound) was also extracted with CER/PCA-NaOH and deionized water to determine the potential hydrolysis of polyP during these extraction processes.

2.4. Analytical methods

To obtain the characterizations of ^{31}P NMR spectrum, 0.01 g of PCA-NaOH extracts, EPS powders and polyP model compound were re-dissolved in 0.2 mL EDTA solution (0.1 mol/L) and 0.2 mL D_2O, followed by the addition of 0.6 mL NaOH solution (1 mol/L). To avoid the transformation of P species, spectra were collected immediately after preparation and the whole process was completed within 2 h. A AVANCE AV400 spectrometer (Bruker Co., Germany) was used and the acquisition parameters were set according to the references (Huang et al., 2015; Zhang et al., 2013). The samples used for XRD analysis were previously dried and calcined in a muffle furnace at 500 °C for 2 h to remove the organic components. The examinations of EPS content, polysaccharides (PS), protein (PN) and humic substances (HS) were carried out as the descriptions in You et al. (2015).

3. Results and discussion

3.1. Effects of CeO_2 NPs on P removal performance

TP removal efficiency in SBBR 1 was maintained at 85.16%, quite comparable with the performance of 86.03% in control. However, with the addition of 20 mg/L CeO_2 NPs, the TP removal efficiency reduced to approximately 59.62%. Additionally, as shown in Fig. A.3, specific P release rate (SPRR) and specific P uptake rate (SPUR) in SBBR 2 dropped steeply to 26.82 and 28.19 mg P/g-biomass, much lower than those in the control (28.22 and 31.35 mg P/g-biomass), leading to the failure of biological P removal.

3.2. Content and distribution analysis of P in the EBPR biofilm

Before the exposure experiments, the fractions and contents of P among SBBR units showed that about 108.5 and 13.3 mg P/g-biomass was reserved in microbial cells and EPS (Table A.2). However, after exposure to 20 mg/L CeO_2 NPs for 3 h (Table A.3), the decreased degradation of complex P (computed via the differences between TP and orthoP) was observed in EPS and a significant amount of orthoP were stored in EPS ($p < 0.05$), which was responsible for the inhibited SPRR in Fig. A.3. Notably, continued exposure to 20 mg/L CeO_2 NPs to 8 h, the distributions of complex P and orthoP in EPS were much higher than the control ($p < 0.05$). While the quantity of PCA-NaOH extractable TP in cells was seriously decreased ($p < 0.05$), which implied the damaged PAOs bioactivity in EBPR system (Huang et al., 2015; Xu et al., 2016).