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Strategies and relative mechanisms to attenuate the bioaccumulation and biotoxicity of ceria nanoparticles in wastewater biofilms

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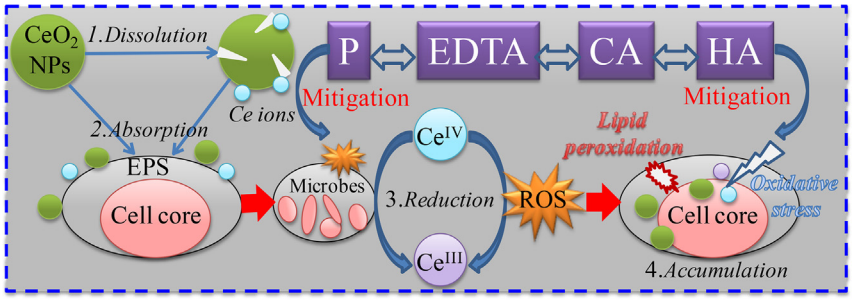
GRAPHICAL ABSTRACT



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ABSTRACT

Inhibitory effects of ceria nanoparticles (CeO_2 NPs) on biofilm were investigated individually and in combination with phosphate (P), ethylene diamine tetraacetic acid (EDTA), humic acid (HA) and citrate (CA) to further explore the toxicity alleviating solutions. Exposure to 20 mg/L CeO_2 NPs significantly decreased the performance of biofilm in nutrients removal. Distribution experiments suggested > 98% of the CeO_2 NPs retained in microbial aggregates, leading to 51.26 μg/L Ce ions dissolution. The dissolved Ce^{IV} and its further being reduced to Ce^{III} stimulated the formation of $O_2^{\cdot -}$ and $^{\cdot}OH$, which increased lipid peroxidation level to 130.93% in biofilms. However, P/EDTA/CA captured or precipitated Ce ions, whereas EDTA/HA/CA shielded NPs-bacteria direct contacts, both disturbing the NPs adsorption, intercepting the redox transition between Ce^{IV} and Ce^{III}, reducing the generation of $O_2^{\cdot -}$ and $^{\cdot}OH$, thus mitigating the toxicity of CeO_2 NPs. These results illustrate the main drivers of CeO_2 NPs biotoxicity and provide safer-by-design strategies.

1. Introduction

In the past decades, the novel properties of engineered nanomaterials were exploited to enhance various technologies and consumer products (Maynard, 2006). Among all, ceria nanoparticles (CeO_2 NPs) are the most promising nanomaterials with increasing applications as catalysts in automotive fuel additives, polishing media and UV absorber (Auffan et al., 2014). The attractiveness comes from their great oxygen storage capacity in relation to the Ce^{III}/Ce^{IV} redox cycle due to the presence of oxygen vacancies, which show dominating technological interests and environmental implications (Marie et al., 2014). The production, utilization and disposal of CeO_2 NPs will inevitably result in environmental releases, and one of the most primary environmental exposure routes of NPs is through wastewater treatment plants (WWTPs) (Gottschalk and Nowack, 2011; Klaine et al., 2008).

As one of the most important bacterial communities in WWTPs,

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biofilm with high densities of cells embedded in self-produced extracellular polymeric substances (EPS) is an effective approach for wastewater decontamination (Flemming and Wingender, 2010). To date, the impacts of CeO_2 NPs on biological nitrogen and phosphorus removal of a sequencing batch biofilm reactor (SBBR) have been studied extensively (Xu et al., 2017a,b; Hou et al., 2015). The potential toxicity mechanisms have been proposed to be relied on the adsorption of CeO_2 NPs onto the biofilm surface, the interaction of NPs with cell membrane and the subsequent internalization into the cells (Xu et al., 2017b; Hou et al., 2015). Additionally, CeO_2 NPs can generate reactive oxygen species (ROS) during its interaction with microbes and during the occurrence of redox process between Ce^{IV}/Ce^{III}, which can result in oxidative stress in microbes and finally induce cytotoxicity (Marie et al., 2014). Though the toxicity effects of CeO_2 NPs on wastewater biological treatment have been comprehensively elucidated, regrettably, few literatures have been published regarding the strategies developed to mitigate the adverse effects of CeO_2 NPs on biological wastewater purification.

To attenuate the risks associated with exposure to CeO_2 NPs during wastewater treatment, understanding their environmental interactions that control NPs transformation is an important aspect (Nowack et al., 2012). The transformations of CeO_2 NPs mediated by environmental factors lead to possible changes in aggregation state, surface composition, reactivity and toxicity potential (Bottero and Wiesner, 2010). Besides, previous studies have shown CeO_2 NPs in aquatic system are characterized by a near-neutral net surface charge, allowing for favorable interaction with the negatively charged surface of bacteria (Barton et al., 2014). Direct contact between CeO_2 NPs and bacterial membranes is considered to favor the reduction of ceria from Ce^{IV} to Ce^{III}, which is associated with the ROS generation and toxicity of CeO_2 NPs to microbial communities (Marie et al., 2014; Thill et al., 2006). Thus, it is necessary to better elucidate the distribution and transformation of CeO_2 NPs in SBBR systems as well as their relationships with nanotoxicity to explore the appropriate approach for toxicity reduction.

It has been reported that exposure to NPs under aerobic conditions would produce excessive reactive molecular species both in vitro and in vivo, which caused the unbalance of cellular redox status and contributed to their toxicities to a variety of cells or microorganisms (Patel et al., 1999; Nel et al., 2006). Nevertheless, the species and kinetics of ROS generation can be differed with different kinds of NPs or environmental factors. Shang et al. (2017a) demonstrated that under UV irradiation, WS_2 and MoS_2 NPs could produce superoxide radical ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$) and hydroxyl radical ($^{\cdot}OH$), whereas CdS NPs only generated $^1O_2$ and $O_2^{\cdot -}$. Furthermore, humic acid (HA) was found to suppress the intensity of $^1O_2$ and $^{\cdot}OH$ produced by the three NPs (Shang et al., 2017a). In addition, bare AgNPs was detected to produce $O_2^{\cdot -}$ and $\cdot OH$ under UV irradiation, while citrate-coated AgNPs only generate $O_2^{\cdot -}$ (Li et al., 2018). However, knowledge concerning the species of ROS produced by microorganisms in response to CeO_2 NPs interactions and their impacts on the development of nanotoxicological outcomes is still lacking. Therefore, it is of great importance to compare and illustrate the effects of proposed toxicity mitigation solutions on the ROS generation.

In this work, phosphate (P), ethylene diamine tetraacetic acid (EDTA), citrate (CA) and HA were introduced and expected to reduce the adverse effects of CeO_2 NPs on SBBR systems. It is hypothesized that: (1) serving as capturing or shielding reagents, P, EDTA, HA and CA would play vital roles in disturbing the bioaccumulation and further dissolution of CeO_2 NPs in biofilm; (2) these added reagents could reduce the generation of ROS, which was associated with the occurrence and transformation of Ce ions; (3) the addition of these reagents would reduce the NPs toxicity by reducing the oxidative stress of CeO_2 NPs on biofilm. To examine these hypotheses, biofilms were exposed to CeO_2 NPs with or without P/EDTA/HA/CA. The individual or combined effects of CeO_2 NPs on subsequent bioavailability and toxicity were detected.

2. Materials and methods

2.1. CeO_2 NPs characterization and biofilm cultivation

Commercially produced CeO_2 NPs was purchased from Sigma-Aldrich (St. Louis, MO), with the particle diameter of less than 50 nm and specific surface area of 30 m^2/g. NPs stock suspension (400 mg/L, pH 7.2 ± 0.2) was prepared according to previous studies (Xu et al., 2017b; Hou et al., 2015). Details about the characteristics of CeO_2 NPs were provided in the E-Supplementary data.

Biofilm cultivation was conducted in a series of SBBR systems with each working volume of 2 L. The combined packing acting as carriers was suspended in the reactors for microorganism attachment. Activated sludge obtained from secondary sedimentation tank was cultured in SBBR with concentration of 3.5 g/L biomass. Synthetic wastewater was then injected into each reactor with the final levels of chemical oxygen demand (COD) 250 mg/L, total nitrogen (TN) 27 mg/L and total phosphorus (TP) 6.25 mg/L (See in the E-Supplementary data). The reactor operation parameters and synthetic wastewater components are identical to those introduced in previous publications (Xu et al., 2017a,b). Batch exposure experiments lasting for 8 h were conducted when SBBR systems reached stable performances in terms of COD, TN and TP removal efficiencies after operated for months.

2.2. Exposure experiment

To perform the exposure experiment, a total of 1.9 L synthetic wastewater and 100 mL NPs stock suspension was introduced into the reactors. The estimated quantities of NPs in wastewater ranged from μg/L to mg/L, while their high affinity for microbial aggregates may induce the accumulation of NPs (Brar et al., 2010; Mohanty et al., 2014). Furthermore, considering the rapid development and growth in CeO_2 NPs application and some other models of entry (i.e., accidental release from a production facility), the potential influences of higher loads of NPs were tested to provide early warning for the NPs risk and find out thorough strategies for attenuation. Therefore, 20 mg/L CeO_2 NPs, which has been investigated and evaluated to inhibit the activity of biofilm (Xu et al., 2017a,b) was chosen as successive and further research in this study. Fresh biofilm inoculated in synthetic wastewater without any CeO_2 NPs and additional reagents is marked as control check (CK).

To mitigate the inhibitory effects of CeO_2 NPs on SBBR performance, capturing and blocking reagents of P, EDTA, HA and CA were added to the wastewater with and without CeO_2 NPs to detect their attenuating toxicity and individual effects on biofilm. P, EDTA and CA with an equimolar amount of CeO_2 NPs were added in the form of $NaH_2PO_4{\cdot}12H_2O$, Na_2EDTA and CA. HA obtained from the International Humic Substances Society was chosen as HA model. HA was dissolved in 0.002 N NaOH in Milli-Q water at 20 mg/L. The HA solution was stirred overnight and filtered through 0.22 μm cellulose acetate membrane (Collin et al., 2014). The total organic carbon (TOC) was measured to be 9.63 mg C/L using a TOC analyzer (Liqui TOC II, Elementar, Germany). Conditions applied to each batch experiment were displayed in E-Supplementary data.

2.3. Fate of CeO_2 NPs in SBBR system

The affinity of CeO_2 NPs for the aquatic and solid phase within the SBBR system was monitored by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500) and visually observed by scanning electron microscopy (SEM, Hitachi S-4800). The distribution of CeO_2 NPs was divided into five parts: dispersed in the wastewater (also considered in association with soluble EPS), in association with loosely bound EPS (LB-EPS), tightly bound EPS (TB-EPS), the microbial pellets without EPS and portions lost in the reactor (including those deposited at the bottom or adsorbed to the inner wall). At the end of the exposure