Effects of CeO2 nanoparticles on the algal composition and photosynthetic activity of phototrophic biofilm

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Abstract. The influences of CeO_2 nanoparticles (NPs) on phototrophic biofilm were explored in terms of the algal composition and photosynthetic activity. Biofilms were cultivated in an artificial flume and exposure experiments were conducted with two levels of CeO_2 NPs (0.05 and 0.5 mg/L) for 10 days. Results showed that 0.05 mg/L CeO_2 NPs treatment altered the algal composition of biofilms, while the total Chl a and the photosynthetic activity maintained at the similar level as the control test. 5 mg/L CeO_2 NPs not only changed the algal composition of biofilms, but also significantly decreased the photosynthetic activity, suggesting that high concentration of CeO_2 NPs might lead to serious consequences to the primary production and nutrient cycling in aquatic environments.

1 Introduction

With the rapidly increasing applications of CeO_2 nanoparticles, they are being unavoidably discharged in fresh waters[1]. The concern about the potential impacts of CeO_2 is increasing due to their toxicity to multiple aquatic organism[2,3]. Toxicity mechanisms are mainly attributed to the generation of ROS, DNA damage and disruption of cell membranes[4].

Phototrophic biofilms are a complex microbial assemblage covering the solid substrates, and ubiquitously distributed in aquatic ecosystem. Algae and cyanobacteria cooccur with heterotrophic bacteria within these biofilms, contributing substantially to primary production and nutrient cycling in aquatic environments[5]. The high sensitivity of biofilms to external pollutions make them as a common agency in natural ecosystems[6]. The contaminants discharged into aquatic environments may affect the microbial activities of biofilms, leading to negative impacts on the function of aquatic ecosystems. Serval researches have been performed to study the potentials of heavy metals on the composition, structure and productivity of biofilms[7]. However, as the amounts of nanomaterials released into aquatic environments are increasing, the potential influence of NPs on the biofilms should be paid more attention. Although the potential impacts of metal NPs on biofilms have been investigated, including metabolic activities and community structure of biofilms[8,9], the potential mechanisms of metal NPs on biofilms remained largely unknown.

Thus, herein, a 10-day exposure experiment was performed to assess the potential impacts of CeO_2 NPs on the algal composition and photosynthetic activity of phototrophic biofilms. Biofilms were cultivated in an artificial hydrodynamic flume located in a greenhouse, and then the mature biofilms were used in the exposure experiments. The

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nanoparticle behaviours in the experimental solutions were determined by dynamic light scattering (DLS), and the algal composition and photosynthetic activity of biofilms were determined by PHYTO-PAM.

2 Materials and Methods

2.1 Characterization of CeO_2 nanoparticles

Commercially available CeO_2 nanopowders were purchased from Sigma-Aldrich (USA) (<50 nm). The nanoparticle stock was prepared with 100 mg CeO_2 NPs suspended in 1L Milli-Q water and then ultrasonicated in bath for 30 min. The hydrodynamic diameter and surface charge were determined by DLS using a Malvern Zetasizer Nano ZSP (U.K). Scanning electron microscope (SEM, Hitachi S-4800 SEM) was used to characterize the size and shape of the CeO_2 NPs. The NP stock solutions were stored at 4 °C in the dark before use.

2.2 Biofilm cultivation and Exposure experiment

Microbial inoculum was scraped from rocks in Xuanwu Lake, Nanjing, East China, and large particles were removed. Then the microbial samples were introduced in an artificial hydrodynamic flume (Figure 1) to cultivate the biofilms. Cobblestones (diameter 3–4 cm) were used as solid substrates for biofilm development. The flume was located in a greenhouse. Evaporation loss was replenished and WC medium was added every 7 days. After five-week cultivation, dense and mature biofilms were used in further experiments.

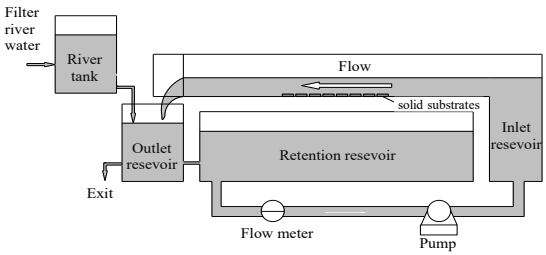

Figure 1. Schematic diagram of the principal experimental flume used for biofilm cultivation.

The exposure experiments were conducted in cylindrical plexiglass microcosms (total volume 6.0 L) described previously. Biofilms on the cobblestones were placed into microcosms that had been filled with 5 L water collected from the incubation flume. Certain amounts of CeO_2 NP stock were introduced to obtain the concentrations of 0, 0.05, and 5 mg/L, respectively. The low level (0.05 mg/L) was selected to represent the environmental relevant concentration in fresh waters, as the concentration of metal nanoparticles (such as TiO_2 and CeO_2 NPs) were predicted within the μg-magnitude in aquatic environments. The high level (5 mg/L) was selected to study the influences of high concentration of CeO_2 NPs, considering that fast-increasing concentrations of released NPs detected in fresh waters. The exposure of the biofilms to NPs lasted for 10 days without any nutrient addition. Then, biofilm samples were collected for further analysis.

2.3 Measurements of the algal composition

To evaluate the influences of NPs on the photosynthetic ability of biofilm, the chlorophyll-a (Chl-a) and quantum yield were determined using the PHYTO-PAM. The machine is equipped with a set of light-emitting diodes that can excite chlorophyll fluorescence at four wavelengths (470, 520, 645, and 665 nm) and automatically classify algae into three types (cyanobacteria, green algae, and diatom). A 5-min dark adaption was conducted prior to the determination. The formula for calculating quantum yield was provided below (1):

$$\text{Quantum yield} = (Fm - F) / Fm \qquad (1)$$

where F is the instantaneous fluorescence measured immediately after the application of a saturating light pulse and Fm is the basal fluorescence.

3 Results and Discussion

3.1 Characterization of CeO_2 NPs

The CeO_2 NP stock was examined using SEM and DLS. The SEM images (Figure 2) illustrated that the NPs had good dispersion, and the average particle size measured by DLS was 82 nm, larger than the advertised particle size (<50 nm). The surface charge of CeO_2 NPs in Milli-Q water was -2.4 mV, with isoelectric point of pH 6.9.

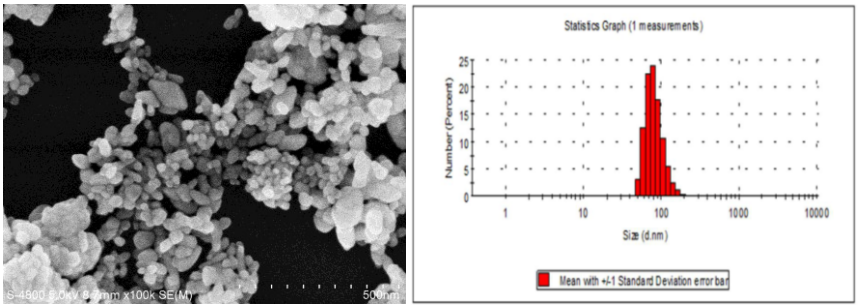

Figure 2. (A) SEM image and (B) particle size distribution of the CeO_2 NP stock suspension measured by DLS.

3.2 Altered biomass in the biofilms

The amount of Chl a and the algal composition of biofilms were determined by PAM, and the results were shown in Figure 3. After 10 days exposure, the total amount of Chl a in the 0.05 mg/L CeO_2 NPs treated biofilms showed a slight decrease compared with the control test ($P>0.05$), while, a significant decrease was observed in the 5 mg/L CeO_2 NPs treated biofilms ($P<0.05$). As for the algal composition, cyanobacteria and diatoms were the dominant algae species in biofilms in the present study. The three kinds of algae exhibited different responses to the CeO_2 NPs exposure. After 10 days exposure to 0.05 mg/L CeO_2 NPs, diatoms showed an obvious reduction compared with the control test ($P<0.05$), whereas, an increase of green algae was observed ($P<0.05$). In the 5 mg/L CeO_2 NPs treatment, the amounts of cyanobacteria and diatoms were both significantly decreased

(P<0.05), and simultaneously the green algae exhibited a significant increase (P<0.05), becoming the dominant algae species in biofilms.

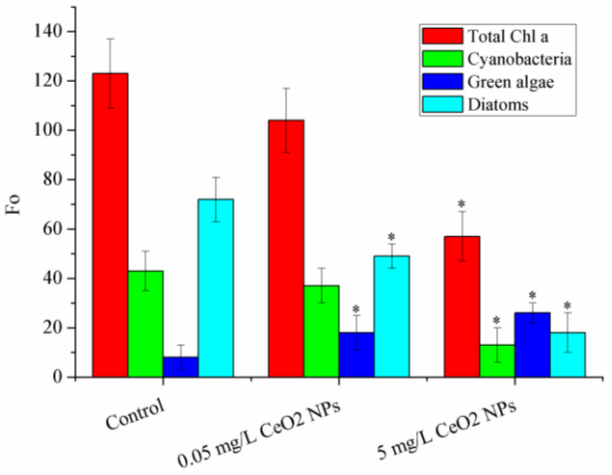

Figure 3. Chlorophyll fluorescence (F0) of Chl a, cyanobacteria, green algae, and diatoms in biofilms exposed to CeO_2 NPs for 10 days. The superscripted * represent significant differences compared with the control test (n = 3).

3.3 Photosynthetic yields of biofilms

Phototrophic biofilm, ubiquitously existing on the surface of solid medium, play an important role in the primary production in aquatic environments. In this study, the photosynthetic yield of biofilms was used to represent the potential capacity of primary production. As shown in Figure 4, the 0.05 mg/L CeO_2 NPs treatment did not change the photosynthetic yield, although the algal composition was obviously altered (Figure 3). These results might be due to that the increased green algae in the 0.05 mg/L CeO_2 NPs-treated biofilms counterbalanced the loss of the photosynthetic activity[10]. Thus, the photosynthetic yield of 0.05 mg/L CeO_2 NPs treated biofilms maintained changeless. However, compared with the control test, 5 mg/L CeO_2 NPs obviously reduced the photosynthetic yield (Figure 4), and changed the algal composition (Figure 3). This high concentration of CeO_2 NPs significantly reduced the physiological activity of algae, resulting in decreases in the photosynthetic yield.

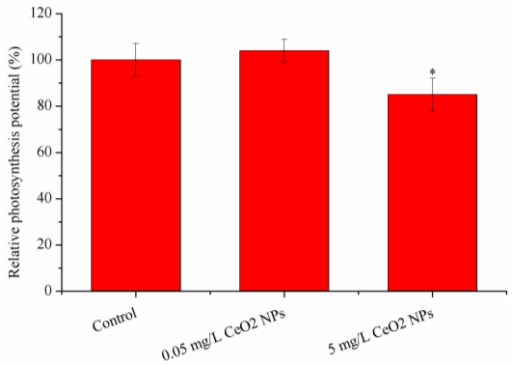

Figure 4. Changes in the photosynthesis potential in biofilms exposed to CeO_2 NPs for 10 days. The superscripted * represent significant differences compared with the control test (n = 3).

4 Conclusion

The effects of CeO_2 NPs on phototrophic biofilm were evaluated in terms of the algal composition and photosynthetic activity. Low level (0.05 mg/L) cerium dioxide nanoparticles altered the algal composition, while no obvious effect was observed on the photosynthetic activity. High level (5 mg/L) cerium dioxide nanoparticles not only changed the algal composition of biofilms, but also significantly decreased the photosynthetic activity.

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