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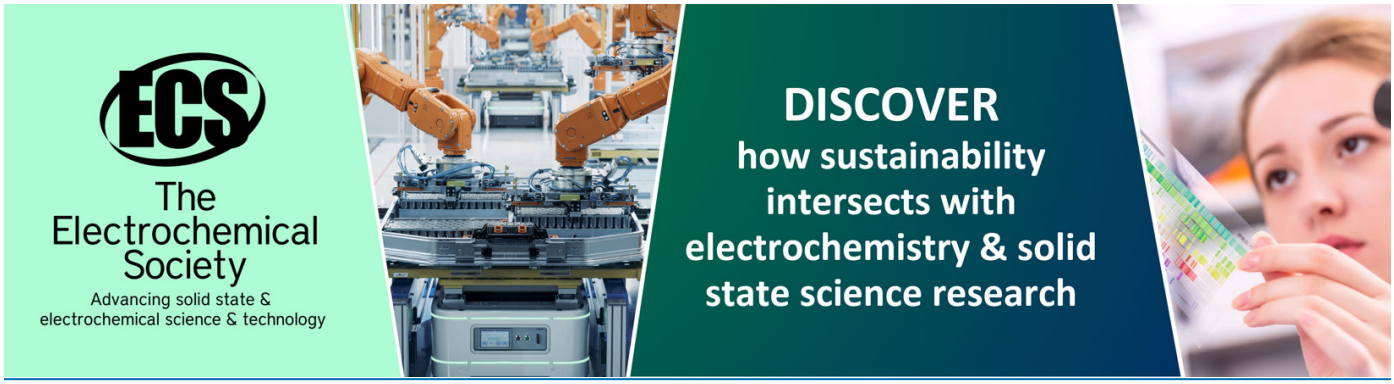

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Effects of Nanoparticle CeO_2 on the Physiology of *Chlorella pyrenoidosa*

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Abstract. Nanoparticle cerium oxide (n-CeO_2) has been widely used, recently, its toxicity to the aquatic environment has received increasing attention. This study aimed to explore the effects of n-CeO_2 on the physiology of *Chlorella pyrenoidosa*. Results showed that n-CeO_2 may inhibited the growth of *Chlorella pyrenoidosa*, and make some influence of chla and protein contents because of the ROS. The activity of SOD and MDA contents also indicated that the high concentration of n-CeO_2 may beyond the range of tolerance, which means ROS content may be a key factor in the toxic effects of n-CeO_2 on *Chlorella pyrenoidosa*.

1. Introduction

With the development of mass production and wide application of nanomaterials, a lot of nanomaterials enter the environment and living organisms cause biosafety and ecotoxicology problems that attracted extensive attention [1-6]. Ceria nanoparticles (n-CeO_2) is one of the most widely used rare earth oxide nanomaterials, which can migrate in the atmosphere, soil and water environment, and through the food chains, causing security problems to individuals, groups and even entire ecosystems [7-11].

Microalgae is a kind of small size single-cell photosynthetic organism, as the primary producer of aquatic ecosystem [12-15], among them, *Chlorella pyrenoidosa* (*C. pyrenoidosa*) is a commonly used toxicity indicator species, and it is also the recommended environmental monitoring test algae species in China [16]. At present, the mechanism of the biological effects of n-CeO_2 on *C. pyrenoidosa* is not clear, and the definition of its toxicity is rather vague [17-20].

By investigating the growth rate, protein contents, chla contents, superoxide dismutase (SOD) activity and malondialdehyde (MDA) contents of n-CeO_2 effect on *C. pyrenoidosa*, explore the possible biological effect mechanism and provide data and scientific basis for assessing the biological effects of n-CeO_2 on algae and its environmental risk accumulation.

2. Materials and Method

2.1. Materials

The species of algae *Chlorella pyrenoidosa* (*C. pyrenoidosa, FACHB-9*) was purchased from the Institute of Hydrobiology at the Chinese Academy of Sciences. *C. pyrenoidosa* was cultured in 100 mL BG-11 medium and placed in an incubators with 25±0.5 °C constant temperature, 5500 lux light intensity with artificial solar light source (light/dark ratio = 12 h:12 h), and carbon dioxide constant aeration (100 mL/min air volume, 2% CO_2).

The nanoparticle cerium oxide (n-CeO_2) (20-50 nm, spherical, 99.5%) was obtained from MACKLIN (a company, shanghai, china). In each experimental, added n-CeO2 into the BG-11 medium and ultrasound for at least 30 min.

2.2. *The determination of cell density*

Take 1 mL samples and dilute to the suitable density, per 24 h. Cell numbers were determined by Hemocytometer under a light microscope (Shanghai CEWEI GUANGDIAN Technology Company, Shanghai, China), using the standard procedure.

2.3. Chlorophyll a content

Determination of chlorophyll a (chla) content in algal cells by using Hot-Ethanol Extraction method. Take 1 mL algal solution and dilute to 5 mL. The diluted solution was put into the plastic centrifuge tube and then centrifuge at 8000 r/min, 4 ℃ for 5 min, after the supernatant discarded. Then added 5 mL absolute ethanol to the centrifuge tube, fully shocked, put the centrifuge tube in a warm water bath (approximately 60 ℃) for 30 min. Supernatants were collected by centrifugation at 8000 r/min, 4 ℃ for 10 min, and measured the absorbance at 652 nm and 665 nm with an ultraviolet spectrophotometer. The chla content was determined using the following equation:

2.4. Protein contents

Protein contents were quantified according to Bradford method with BSA as standard at 595nm. The extraction process of the protein contents was as followed: 10 mL of the algae suspension sample was put into the plastic centrifuge tube and then centrifuged at 8000 r/min, 4 ℃ for 5 min, and removed the supernatant. Then, added 10 mL phosphate buffer (PH=7.8 0.05 mM) into each tube, crushed by sonicator in the ice bath for 10 min (broken 5 s, interval 5 s), centrifuged the homogenates at 8000 r/min, 4 ℃ for 10 min. The supernatant is the crude enzyme which used to analyse the protein contents.

2.5. *SOD activity and MDA contents*

SOD was studied as an enzymatic antioxidant. The activity of SOD was measured by Nitroblue Tetrazolium photochemical reduction reaction (NBT method). MDA was studied as an in vitro marker of lipid peroxidation. The MDA contents were measured by thiobarbituric acid colour reaction (TBA method). The extraction of the SOD and MDA were the same to section 2.5.

3. Result and discussion

3.1. Effects of n-CeO_2 on algal growth

The growth curves of *C. pyrenoidosa* were presented in Fig. 1.

According to the Fig. 1, during 168 h, no matter the concentrations of n-CeO_2, the algae density decreased compared with the control group, with the increasing of the concentration of n-CeO_2, the cells density decreased, n-CeO_2 produced negative impacts on the algal growth and inhibited the cells growth significantly. Also, with the n-CeO_2 concentration increasing, the increasing the average rate of *C. pyrenoidosa* was decreased. The experimental group with 100 mg/L of n-CeO_2 has the cell density with 70.40×10^6 cells/mL, which is the lowest concentration among the experimental group, compared the

control group is 98.88×10^6 cells/mL, which means decreased 28.80%. The n-CeO_2 can cause shading effect, aggregation effect and toxic ions (Ce^{3+}), which may change the *C. pyrenoidosa* in in pigment contents, protein contents or enzyme activity, thus will producing toxic effects on the growth of microalgae [21].

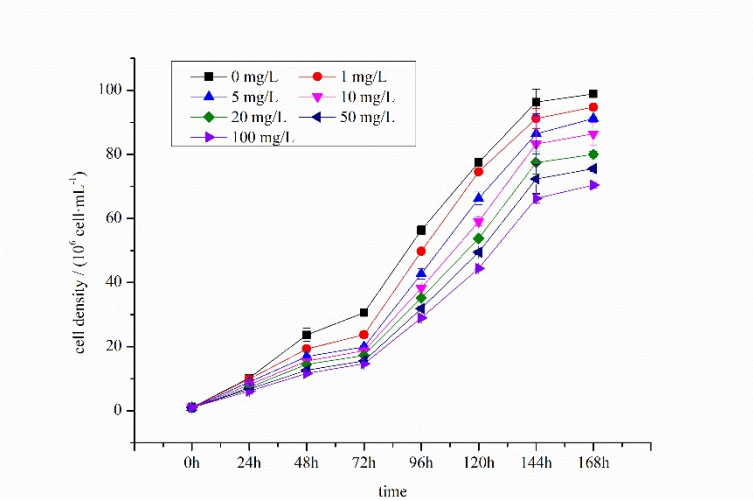

Figure 1. The Change of Biomass of the Mutation and Start Algae Strains

3.2. *Effects of n-CeO_2 on chla contents of C. pyrenoidosa*

The effects of n-CeO_2 on chla contents at 168 h are shown in Fig. 2 Results indicate that the different concentrations of n-CeO_2 compared with the control group had a significant impact on chla contents of *C. pyrenoidosa*. Then chla contents of *C. pyrenoidosa* more highly than that of the experimental group. The reduction of chla is a commonly observed symptom of toxicity in algae [22]. Xingxing He [23] indicated that, ThO_2 NPs has the impact on the *C. pyrenoidosa*, at 200 μm, ThO_2 NPs will make the concentration of chla decreased about 47.52% compared with the control group; Yu Zhen [24] measured the photosynthetic pigments about *Chlamydomonas reinhardtii* of algal cultures exposed to some different nanomaterials and show there are the similar trends as algae growth were observed in total chlrophyll.

The results indicated that the low concentration of n-CeO_2 can promote the synthesis of chla of *C. pyrenoidosa* and accelerate its growth, while the high concentration may lead to the formation of a large number of ROS which may damage the structure and function of pigment molecules or inhibit their anabolism. These can explain the phenomenon that at the low concentrations, the contents of chla were higher than the high concentrations from Fig. 3.

3.3. Effects of n-CeO_2 on protein contents of C. pyrenoidosa

Soluble protein is an important osmotic regulator and nutrient in biological cells, and plays a protective role in cell life activities. The effects of n-CeO_2 on protein contents at 168 h are shown in Fig. 3. According to the results, with the increasing of the concentration of n-CeO_2, the protein contents decline significant at first, the experimental group with 20 mg/L n-CeO_2 has the lowest protein contents about 0.47 mg/10^6 cells, however the group with 100 mg/L is highest about 1.81 mg/10^6 cells. What's more, at the concentration of 1 mg/L, the protein contents almost same to the control group. Which may indicate that *C. pyrenoidosa* accumulates ROS, cause algal cells to resist this threat by increasing the content of antioxidant enzymes, consistent to the chla. As A. Xiao [25] showed that different nanomaterials will make the imparity influence on the protein, with the increasing concentration of N, S doped CQDs, the protein content decreasing, however the low concentration of CdS QDs may increasing protein concentration. Few studies suggested that high concentrations of nanomaterials may also lead to the higher protein contents, as showed in this paper.

3.4. Effects of n-CeO_2 on enzymatic activities of C. pyrenoidosa

The effect of n-CeO_2 on the activity of SOD and MDA contents of *C. pyrenoidosa* at 168 h were shown in Fig. 4 and Fig. 5.

SOD (superoxide dismutase) is an important active oxygen protective enzyme, organisms, with peroxidase, catalase and glutathione constitute the peroxidation defense system, which catalyse disproportionation of ROS in biological cells, remove ROS effectively and prevent the peroxidation of cell membrane system: generated hydrogen peroxide, and then hydrogen peroxide enzyme into harmless molecules of oxygen and water. MDA (malondialdehyde) is the production of membrane lipid peroxidation, which has been widely used as an index to measure the damage of membrane lipid peroxidation, because of the environmental stress, ROS balance in organisms is broken, the excessive ROS will cause membrane lipid peroxidation and produce MDA.

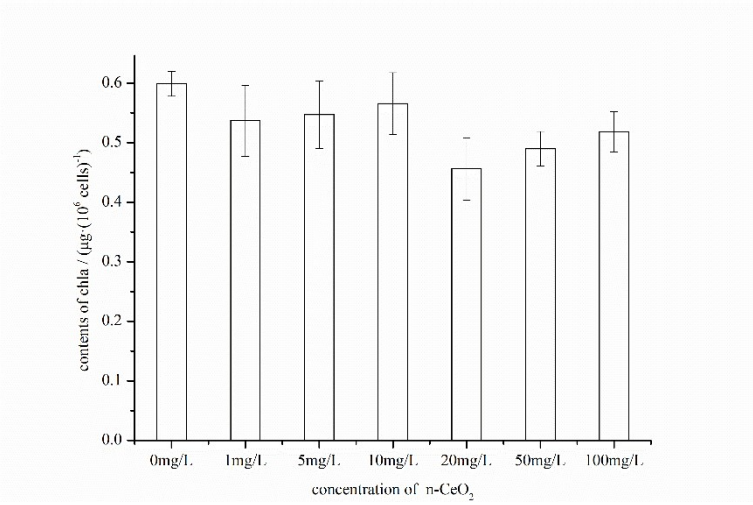

Figure 2. The Comparison of chla contents

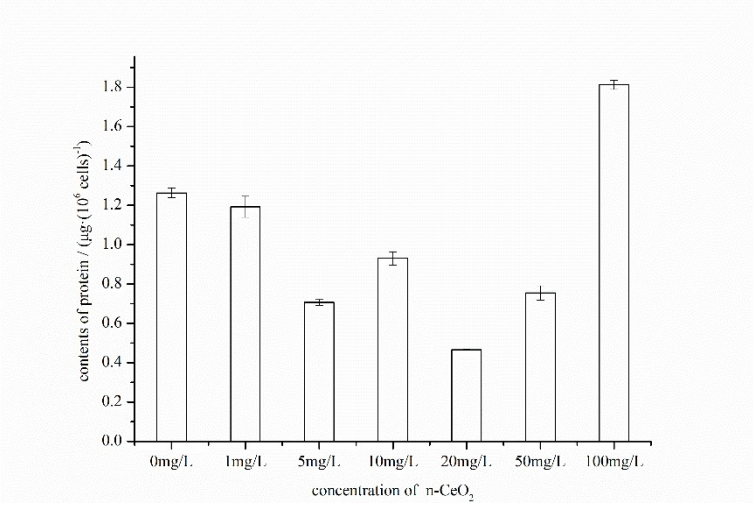

Figure 3. The Comparison of protein contents

As shown in the Fig. 4, the experimental group has higher activity of SOD than the control group, this indicated that oxidative stress is increased by the n-CeO_2. What's more, when the concentration of n-CeO_2 at 100 mg/L the SOD activity decreased that shows ROS damage to algal cells may beyond the range of self-regulation, which can proved by the phenomenon shows in the Fig. 5, the contents of MDA decreased at the low concentration while it increased at the high one. This phenomenon is similar as

Elisabetta Morelli [26] indicated, when *Dunaliella tertiolecta* grown for 96 h in sea water added with CdSe/ZnS, MDA contends dropped first and then rised.

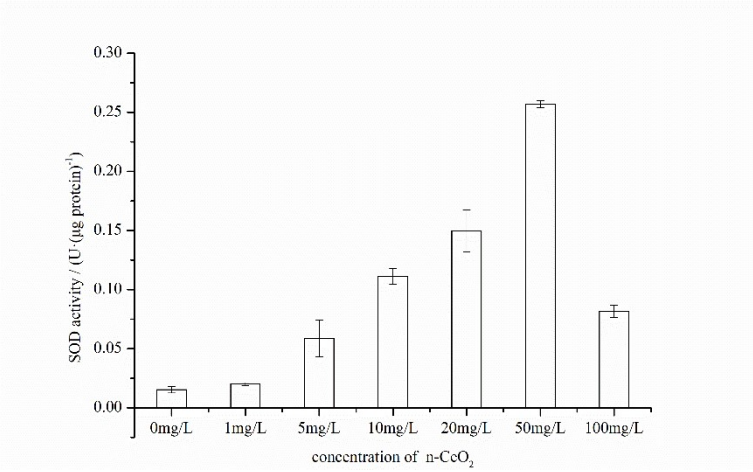

Figure 4. The Comparison of SOD activity

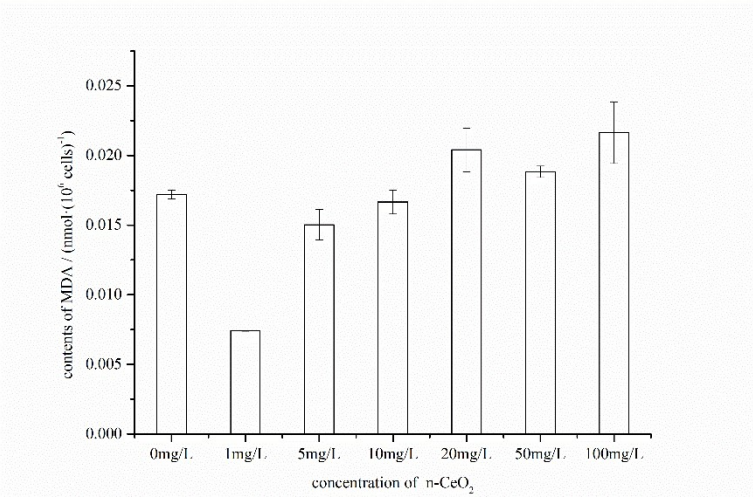

Figure 5. The Comparison of MDA contains

4. Conclusion

The research used *C. pyrenoidosa* as a model organism to evaluate the potential environmental risks of n-CeO_2. In the experimental, we investigated the growth inhibition, Chla contents, protein contents, the activity of enzymatic. The surveys demonstrated that n-CeO_2 can affect the growth rate of algae cells, and the content of Chla and protein will change due to the large amount of ROS production, furthermore, the activity SOD and MDA contents show that the high concentration of n-CeO_2 may beyond the range of tolerance lead the lower activity SOD and lower MDA contents, which means promote the production and accumulation of ROS, is an important for n-CeO_2 biological effects.

Acknowledgments

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