Ecotoxicology and Environmental Safety 132 (2016) 353–359

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of sodium pentaborate pentahydrate exposure on *Chlorella vulgaris* growth, chlorophyll content, and enzyme activities

Xueqing Chen, Yuansheng Pei *

The Key Laboratory of Water and Sediment Sciences, Ministry of Education, School of Environment, Beijing Normal University, No. 19, Xinjiekouwai Street, Beijing 100875, China

ARTICLE INFO

Article history:
Received 6 March 2016
Received in revised form
13 June 2016
Accepted 17 June 2016
Available online 28 June 2016

Keywords:
Sodium pentaborate pentahydrate
Characterization
Chlorella vulgaris
Growth rate
Chlorophyll
Enzyme

ABSTRACT

Sodium pentaborate pentahydrate (SPP) is a rare mineral. In this study, SPP was synthesized from boric acid and borax through low-temperature crystallization, and its effects on the growth of the alga, *Chlorella vulgaris* (*C. vulgaris*) were assessed. The newly synthesized SPP was characterized by chemical analysis, X-ray diffraction, Fourier-transform infrared spectroscopy, Raman spectroscopy, thermogravimetric analysis, and differential thermal analysis. The changes in *C. vulgaris* growth, chlorophyll content, and enzyme activities upon exposure to SPP for 168 h were evaluated. Results showed that SPP treatment was detrimental to *C. vulgaris* growth during the first 24–120 h of exposure. The harmful effects, however, diminished over time (168 h), even at an effective medium concentration of 226.37 mg B L^{-1} (the concentration of boron applied per liter of culture medium). A similar trend was observed for chlorophyll content (chlorophyll *a* and *b*) and indicated that the photosynthesis of *C. vulgaris* was not affected and that high levels of SPP may even promote chlorophyll synthesis. Superoxide dismutase and catalase activities of *C. vulgaris* increased during 24–120 h exposure to SPP, but these activities gradually decreased as culture time progressed. In other words, the initial detrimental effects of synthetic SPP on *C. vulgaris* were temporary and reversible. This research provides a scientific basis for applications of SPP in the environment.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Sodium pentaborate pentahydrate (SPP), also known as sborgite, is a rare natural mineral found only in Larderello, Italy (Merlino and Sartori, 1972), and California, USA (Gong et al., 2014). SPP can be used as a component of enzymatic liquid detergents (Tai, 1983) and as a feed additive to prevent viral and bacterial pathogen contamination in shrimp (Perez, 2012). It can also be used as a pharmaceutical ingredient for enhancing cutaneous wound healing rates (Doğan et al., 2014). Owing to its low packing density, SPP shows great potential in applications such as drug targeting and energy harvesting (Hu et al., 2001; Kwon et al., 2012). However, no research has been reported on the environmental effects of SPP. With the increasing number of potential applications of SPP, its dispersal into the environment through domestic sewage and industrial waste is most likely inevitable. It is therefore essential to determine the effects of SPP on the environment and particularly on aquatic ecosystems.

Algae are the primary producers in the aquatic ecosystems and form the base of the aquatic food chain. The diversity and biomass of algae both directly influence the structure and function of the aquatic ecosystems and play an important role in the stabilization and equilibrium of the ecosystems (Qian et al., 2012). Algae species are also widely used in research on the response of aquatic ecosystems to chemicals, mainly due to their characteristics of small size, rapid propagation, and sensitivity to toxicants (Oukarroum et al., 2012). *Chlorella vulgaris* (*C. vulgaris*) is a useful test subject in the toxicity studies of water-soluble substances, metals, and other pollutants (Qian et al., 2009; Rashkov et al., 2012). To date, however, there has been no study on the effects of SPP exposure on *C. vulgaris*.

For this study, owing to the unavailability of commercial SPP product (Zhang et al., 2014), SPP was generated directly from the reaction of boric acid and borax, using an improved synthetic method. This SPP product was characterized by chemical analysis, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, thermogravimetric analysis (TGA), and differential thermal analysis (DTA). The effects of this SPP product on a series of physiological and biochemical parameters of *C. vulgaris*, including growth rates, chlorophyll content, and superoxide dismutase (SOD) and catalase (CAT) activities were evaluated. This research helps to understand how SPP affects *C.*

* Corresponding author.
E-mail address: yspei@bnu.edu.cn (Y. Pei).

http://dx.doi.org/10.1016/j.ecoenv.2016.06.024
0147-6513/© 2016 Elsevier Inc. All rights reserved.

vulgaris, which could provide basic data for aquatic ecology risk assessments of SPP exposure.

2. Materials and methods

2.1. Chemicals

All the chemicals used in this research were analytical grade. H_3BO_3 and $Na_2B_4O_7 \cdot 10H_2O$ were purchased from Xilong Chemical Co., Ltd.

2.2. Synthesis of SPP

SPP was synthesized according to the methods reported by Nies and Hulbert (1967), Perez and Pablo (2005) with slight modification. In summary, a given quantity of boric acid and borax were added to 0.80 L of purified water (B_2O_3/Na_2O mole ratio 4.2). In the dissolving process, the mixture solution was kept at 60 °C and stirred by a magnetic stirrer. After the boric acid and borax dissolved completely, the hot solution was filtered through a Double Circles filter paper No. 202 (GE Bio-Sciences [Hangzhou] Co., Ltd) in a Buchner funnel. Then, the filtrate was transferred to a plastic beaker together with the stirrer. When the solution cooled down to 34 °C, the beaker was transferred to an ice-water bath until the temperature dropped to 10 °C. The mixture was stirred during the cooling process to promote crystal formation. The mixture was then refrigerated at approximately 4 °C for 18 h. After that, the crystals were collected in a Double Circles filter paper No. 103 in a Buchner funnel. Finally, the product was dried in an oven at 35 °C until its weight became constant.

2.3. Characterization of the product

The concentration of B_2O_3 in the product was analyzed by mass titration in the presence of mannitol by using an NaOH standard solution as titrant and phenolphthalein as the indicator; Na_2O was analyzed using an HCl standard solution as titrant. The physical characteristics of the product were examined by X-ray diffraction (XRD) (recorded by an X pert pro MPD diffractometer with CuKα radiation), Fourier transform infrared spectroscopy (FT-IR) (recorded over the 400–4000 cm^{-1} region on a NEXUS 670 spectrometer with KBr pellets at room temperature), Raman spectroscopy (recorded over the 300–1800 cm^{-1} region on a LabRAM Aramis Raman spectrometer), thermogravimetric analysis (TGA), and differential thermal analysis (DTA) (performed on an HTC-3 thermal analyzer in N_2 atmosphere with a heating rate of 10 °C min^{-1}).

2.4. Algae culture

The microalgae *C. vulgaris* (FACHB-8) was purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences. The algae were cultured in OECD medium (OECD, 2006). To eliminate other boron sources, the algae used were cleaned three times with boron-free OECD medium and cultured at 25 ± 1 °C in 250 mL boron-free flasks (4110, Nalgene) containing 100 mL boron-free OECD medium. The algae were illuminated with cool-white fluorescent light (100 ± 5 uE m^{-2} s^{-1}) with daily cycles of 14 h light and 10 h dark. Both the culture medium and flasks were sterilized at 121 °C, 0.1 MPa for 30 min. For cell experiments, 30 mL of *C. vulgaris* was added to 270 mL of the boron-free OECD medium (initial cell concentration approximately 10^5–10^6 cells mL^{-1}) containing different boron concentrations (provided by SPP) until the desired concentrations were reached.

2.5. Exposure protocol

For the purpose of comparison, levels of SPP were expressed in terms of B equivalents based on the fraction of boron on a molecular weight basis. So "mg B L^{-1}" was expressed as the concentration unit of SPP in culture medium. A stock solution of 200 mg B L^{-1} SPP was prepared by dissolving the product in boron-free OECD medium. The working solutions were obtained by dilution of the stock solution to the desired concentrations required for the experiments. The final concentrations of the working solutions were 1.0, 10, 20, 40, and 80 mg B L^{-1}. Flasks without the stock solution were used as the control group, and three replicates were made for each experiment. Growth rates, chlorophyll contents, and SOD and CAT activities were measured at 24, 48, 72, 96, 120, and 168 h.

2.6. Algae growth rate assays

Algae cultured in boron-free OECD medium (20 mL) was diluted to double series. Each diluted algae cell density was determined using a hemocytometer under a light microscope (Olympus BX 41, Japan), and the cell optical density (OD) was measured on a spectrophotometer (DR-6000, HACH) at 680 nm against boron-free OECD medium. A good linear relationship between cell numbers and OD values was observed; the relationship was determined as $y=39.911x+0.0756$ ($R^2=0.998$), where y represented cell density (10^6 mL^{-1}) and x represented OD at 680 nm. The OD value measured at 680 nm against boron-free OECD medium indirectly reflected algae growth (Kong et al., 2010).

In each experiment, algae cell medium (10 mL) was collected by centrifugation (10,000 g for 10 min at 4 °C) and washed three times with sterile distilled water. The collected algae samples were suspended in 10 mL boron-free OECD medium. The inhibition efficiency was calculated using the following formula (Xiong et al., 2014):

$$I = \frac{C_0 - C_t}{C_0} \times 100$$

where I is the inhibition efficiency (%), and C_0 and C_t represent average algae cell density in the control and treated groups, respectively.

Medium effective concentration (EC_{50}) values for algae exposure tests and their 95% confidence intervals were calculated using SPSS probit analysis of transformed SPP concentration as natural logarithm data against inhibition efficiency.

2.7. Chlorophyll content analysis

Algae cell medium (20 mL) was centrifuged at 10,000 g for 10 min. The harvested algae samples were suspended by 5 mL of *N,N*-dimethylformamide in the dark at 4 °C for 24 h. Samples were then centrifuged at 5000 g for 10 min. The OD values of the supernatants were measured at 647 and 664.5 nm against *N,N*-dimethylformamide, treated in the same way. The following formulae (Inskeep and Bloom, 1985) were used for calculation of chlorophyll *a* and chlorophyll *b* content:

$$\text{chlorophyll } a = [12.7 \times OD_{664.5}] - [2.79 \times OD_{647}]$$

$$\text{chlorophyll } b = [20.7 \times OD_{647}] - [4.62 \times OD_{664.5}].$$

2.8. Determination of SOD and CAT enzyme activities

To obtain enzyme solutions from algae cells, 20 mL of algae cell medium was centrifuged at 10,000 g for 10 min at 4 °C. The harvested algae samples were suspended in 1 mL of 0.05 M

Download English Version:

https://daneshyari.com/en/article/4419116

Download Persian Version:

https://daneshyari.com/article/4419116

Daneshyari.com