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Effect of TiO_2 nanoparticle aggregation on marine microalgae *Isochrysis galbana*

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ABSTRACT

TiO_2 nanoparticles (NPs) could adversely impact aquatic ecosystems. However, the aggregation of these NPs could attenuate this effect. In this work, the biological effects of TiO_2 NPs on a marine microalgae *Isochrysis galbana* were investigated. The aggregation kinetics of TiO_2 NPs under different conditions was also investigated to determine and understand these effects. Results showed that, though TiO_2 NPs had no obvious impact on the size and reproducibility of algal cells under testing conditions, they caused a negative effect on algal chlorophyll, which led to a reduction in photosynthesis. Furthermore, fast aggregation of TiO_2 NPs occurred under all conditions, especially at the pH close to the pH_{zpc}. Increasing ionic strength and NP concentration also enhanced the aggregation rate. The aggregation and the following sedimentation of TiO_2 NPs reduced their adverse effects on *I. galbana*.

Introduction

Nanoparticles (NPs) display unique physical and chemical characteristics such as large specific surface areas and unique surface structures that cause high surface reactivity. They are increasingly used for industrial and commercial purposes as catalysts, semiconductors, cosmetics, microelectronics, drug carriers, *etc.* The production of engineered NPs is expected to be 58,000 tons in 2020 (Mayland, 2006). The wide application of NPs has increased significant concerns about their environmental release and their potential toxicity to aquatic organisms such as marine phytoplankton (Miao et al., 2010; Matranga and Corsi, 2012; Miller et al., 2012).

TiO_2 NPs have been widely used in many commercial products (including self-cleaning and antimicrobial coatings/paintings, cosmetics, and sunscreens) because of their high chemical reactivity and broad UV attenuation properties (Maier, 2005; Chen, 2007). The direct release of TiO_2 NPs from urban applications to an aquatic system could achieve concentrations in μg/L (Kaegi, 2008). TiO_2 NPs can produce reactive oxygen species (ROS) which may induce oxidative damage to bacteria (Adams et al., 2006), fresh water invertebrate (Lovern and Klaper, 2006), and different cell types (Long et al., 2006; Rothen-Rutishauser et al., 2006).

The toxicity of NPs not only depends on their total concentration but also depends on their aggregate size and surface chemistry (Grassian, 2008; Gao et al., 2009). The size of aggregates was a key factor in determining their uptake and effect on cells (Rothen-Rutishauser et al., 2006), as well as their bioavailability to plant roots, algae, and fungi (Navarro

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et al., 2008a). Therefore, understanding the fate and transport of TiO_2 NPs in water was essential for determining their availability and toxicity on aquatic organisms.

Surface potential dominates the aggregation of NPs. In addition, pH, ionic strength, and cation valence affect the aggregation (Dunphy Guzman et al., 2006; French et al., 2009). Moreover, the presence of organic acid such as fulvic acid decreases TiO_2 NP aggregation (Domingos et al., 2009). The fastest aggregations were also observed at a pH close to the pH of zero point of charge (pH_{zpc}) under all conditions (Dunphy Guzman et al., 2006; Pettibone et al., 2008; Domingos et al., 2009; French et al., 2009).

Although the ecotoxicity of TiO_2 NPs in freshwater has been well studied, the effect of TiO_2 NPs on marine algae, a primary producer in marine ecosystem, has not been well understood. The objective of this research was to determine the effect of TiO_2 NPs on a marine microalgae, *Isochrysis galbana*. In addition, because the aggregation of NPs could significantly influence NP toxicity (Hotze et al., 2010), the aggregation kinetics of TiO_2 NPs were also investigated to develop insights into both the direct and indirect biological effects of NPs on a marine ecosystem. It was also important to assess the environmental risks related to the release of the NPs (Dale et al., 2015).

1. Materials and methods

1.1. NPs and chemicals

TiO_2 NPs were purchased from Skyspring Nanomaterials Inc. (USA), Product #: 7930DL. It had a purity of 99.9%, with an advertised main size of 5 nm. Its crystal was anatase, with a specific surface area greater than 150 m^2/g. Millipore water was produced by using a Synergy® ultrapure water system (Millipore, USA). NaOH (99%) and HNO_3 (67%) were purchased from Fisher Scientific (USA).

1.2. Preparation of NP suspension for toxicity tests

TiO_2 NP suspensions for toxicity tests were prepared by diluting a NP stock solution with Guillard f/2 culture medium (Guillard, 1975). The seawater of the f/2 culture medium was obtained from the Southern Ocean near Antarctica, with a sanity of 34‰, and then filtered through a 0.22 μm membrane filter and sterilized. The stock solution of TiO_2 NPs (1000 mg/L) was made by adding 0.1000 ± 0.001 g of TiO_2 NPs into 100 mL of the f/2 culture medium. This stock solution was sonicated for 10 min at 25 Hz in a water bath prior to use. Different volumes of this stock solution were added to the blank f/2 culture medium to create testing TiO_2 NP suspensions with concentrations of 0, 40, 100, 200, 400, 1000 mg/L, respectively. The toxicity test solution was prepared by mixing this testing TiO_2 NP suspensions with algae at a volume ratio of 1:1, so that the highest TiO_2 NP concentration in the toxicity test was 500 mg/L. While the environmentally relevant concentration of NPs was generally at μg/L level, the high concentration used in this research reflected a scenario when a concentrated waste stream was accidentally discharged into the near shore water in short-term. The change of pH for these TiO_2 NP suspensions during the experiment was less than 0.5 pH unit (from 7.55 to 8.01) during the experiment, meeting the OECD guideline for this type of experiment (OECD, 2011).

1.3. NP influence on algal growth and photosynthesis

I. galbana was provided by the Institute of Oceanology, Chinese Academy of Sciences (IOCAS, China). It was incubated using a f/2 culture medium in a lab several months prior to testing. Algae, with an initial cell density of 2.35×10^5 cells/mL, were used in all tests. The growth inhibition tests of *I. galbana* were conducted according to OECD guidelines for testing chemicals (OECD, 2011). Four milliliters of algal solution and 4 mL of TiO_2 NP suspension were transferred into each of the 17 × 100 mm polystyrene culture test tubes (Fisher, USA). All test tubes were then incubated at 24°C, under a light/dark ratio of 12 hr:12 hr, with an illumination of 4000 lx during the light on period. After 24, 48, 72, and 96 hr, the reference fluorescent units (RFUs) of the sample were detected by using a Trilogy fluorescent meter (Turner, USA). RFU represented a reference value of intravital chlorophyll, which indicated chlorophyll changes inside algae cells and could be used as an indicator of algal growth status. The culture medium and TiO_2 NP suspensions have different background RFUs, hence, the RFU of the sample was corrected by deducting the background RFU of the test tube from the total RFU. All tests were conducted in duplicate, and the culture tubes were shaken once at a time interval of 24 hr to re-suspend algae and NP aggregates.

A micro-respiration (MR) system (Unisense, Denmark) was employed to determine the photosynthesis of the samples after 96 hr of incubation. Samples were carefully transferred into a specially designed MR glass chamber (containing a stirrer) and then sealed with a special cap. The cap contained a hole through which the MR-oxygen sensor could be inserted into the chamber. The chamber volume was previously determined to be 2146 μL by using the method provided by the manufacturer. Then, the MR system (including its chamber, rack, stirrer, and oxygen sensor) was placed in a transparent Perspex water bath in an incubator, at a temperature of 24 ± 0.1°C, with a light intensity of 4000 lx. The oxygen concentration inside the MR chamber was measured and the photosynthesis was indicated by the rate of oxygen generation that was auto-calculated by the MicOx program. The cell density and size were measured using a Moxi Z mini automated cell counter (Orflo, USA).

1.4. Shading effect of TiO_2 NP suspensions

The TiO_2 NP suspension could physically block light and impact algae activity, even when there was no direct contact between them. In order to identify the effect of TiO_2 NPs on algae without any physical contact between them, a shading experiment was conducted. A test tube (with the same algae density as the algae control in a biological assay) was inserted in a customized 50 mL glass tube that contained 20 mL of 500 mg/L freshly prepared TiO_2 NP solution (resuspended every 24 hr). This was to avoid any contact by the algae with the NPs (illustrated in Fig. 1). The RFU, cell density and size, and the rate of oxygen generation of the algae, were measured after 96 hr. This test was also conducted in duplicate.

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