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Method Article

Methods of determining titanium dioxide nanoparticles enhance inorganic arsenic bioavailability and methylation in two freshwater algae species

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A B S T R A C T

We developed the effect of titanium dioxide nanoparticles (nano-TiO_2) on the bioaccumulation and biotransformation of arsenic (*As*), which remain largely unknown. We thus exposed two freshwater algae (*Microcystis aeruginosa* and *Scenedesmus obliquus*) to inorganic *As* with the aim of increasing our understanding on *As* bioaccumulation and methylation in the presence of nano-TiO_2. Direct evidence of TEM and EDX image showed that nano-TiO_2 (anatase) entered the exposed algae. Thus, nano-TiO_2 as carriers boosted arsenic accumulation and methylation in these two algae species, which varied with both inorganic *As* speciation and algae species. Specifically, nano-TiO_2 could enhance markedly arsenate accumulation in *M. aerugginosa* and arsenite accumulation in *S. obliquus*. Similarly, we found higher content of *As* methylation in *M. aeruginosa* of arsenite with 2 mg L^{-1} of nano-TiO_2 treatment and in *S. obliquus* of arsenate treatment. Additionally, *S. obliquus* exhibited higher *As* methylation compared to *M. aeruginosa*, being more sensitive to *As* associated with nano-TiO_2 than *M. aeruginosa*. Due to changes in pH levels inside these exposed algae, the *As* dissociation from nano-TiO_2 inside algal cell enhanced *As* methylation. Accordingly, the potential influence of nanoparticles on the bioaccumulation and biotransformation of their co-contaminants deserves more attention.

- Nano-TiO_2 entry is assumed to promote *As* accumulation into exposed algae.
- Nano-TiO_2 had different carrying capacities for different forms of *As* and algae.
- *As* dissociation from nano-TiO_2 is assumed to enhance *As* methylation in algae.

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Name and reference of original method	*If applicable, include full bibliographic details of the main reference(s) describing the original method from which the new method was derived.*
Resource availability	If applicable, include links to resources necessary to reproduce the method (e.g. data, software, hardware, reagent)

Method

Nano-TiO_2 and arsenic preparation

We used the anatase form of nano-TiO_2 purchased from the Sigma-Aldrich Corporation with a particle size of less than 25 nm and a purity of > 99.7%. A nano-TiO_2 stock suspension (1 g L^{-1}) was prepared by first suspending nanoparticles in ultrapure water. We then sonicated the solution at 33 W for 30 min. The average hydrodynamic size of nano-TiO_2 was 193 ± 10 nm, as measured by the dynamic light scattering technique (DLS, Malvern Instruments, UK) at automatic attenuator mode. Experimental nano-TiO_2 concentrations of 100 μg L^{-1} and 2 mg L^{-1} were diluted from the stock suspension. The aggregate morphology of nano-TiO_2 was observed by a scanning electron microscope (SEM, S-4800, Hitachi, Japan). We used $Na_3AsO_4 \cdot 12H_2O$ and $NaAsO_2$ to prepare *As* stock solutions at 1 mM, which were stored at 4 °C in the dark until further use. Additionally, we measured the average hydrodynamic diameter (d_H) and zeta potential (ζ) using DLS, and pH levels of nano-TiO_2 in BG-11 culture media with *As*(III) and *As*(V) of 10 μM at 0.1 mg L^{-1} and 2 mg L^{-1} TiO_2 concentrations.

Exposed algae species

The two freshwater alga species (*S. Obliquus* and *M. Aeruginosa*) used in our experiments were inoculated under sterile conditions in BG-11 media in Erlenmeyer flasks at 25 °C. The light-dark cycle used was 16:8 with a light intensity of 115 μmol photons m^2 s^{-1}. For the following experiments, exposed algae were shaken at 100 rpm using a shaker to avoid settling.

Algae toxicity and stress

Toxicity of *As*(III) and *As*(V) was determined using 96 h growth rate bioassays. Algal cell at the exponential growth phase were added separately into a final concentration of *As*(III) (10 μM) and *As*(V) (10 μM) under increasing nano-TiO_2 levels (from 0 to 200 mg L^{-1}). The initial cell density of exposed algae was 10^6 cells mL^{-1}. We used a hemocytometer and a microscope to measure algal cell density every 24 h until the end of the exposure experiment. The specific growth rate (μ) of cells was thus calculated according to the method reported by Zeng et al. [1]. Afterwards, the 96 h EC50 was calculated based on μ values of tested algal cells using a probability unit graphical method [2].

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