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Biomethylation and volatilization of arsenic by the marine microalgae *Ostreococcus tauri*

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HIGHLIGHTS

- *O. tauri* could tolerate 100 μM As(III) or As(V).
- *O. tauri* is able to biomethylate and biovolatilize As.
- As(V) reduction was speculated to be the rate-limiting factor for As methylation and volatilization.

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ABSTRACT

Ostreococcus tauri is a marine green microalga, recognized as a model organism of the marine phytoplankton assemblage and widely distributed from coastal to oligotrophic waters. This study showed it could tolerate both arsenite and arsenate concentrations of up to 100 μM, and cellular As concentration increased significantly ($P < 0.01$) with increasing concentration of As(V) in the medium (0–50 μM). It was revealed that As biotransformations were mediated by algal cells. Volatilized As was detected and the ability of As biovolatilization by *O. tauri* was demonstrated. The reduction of As(V) to As(III) might be the limiting step for As methylation and volatilization from seawater since the treatment with As(III) yielded five times more volatile As as compared to that with As(V). Arsenic biogeochemical cycle in the marine environment might play an important role based on the huge surface area of ocean (71%) and the massive number of marine phytoplankton.

1. Introduction

Arsenic (As) is highly toxic and broadly distributed in the lithosphere, hydrosphere, atmosphere and biosphere (Ng, 2005; Bhattacharya et al., 2007). The transport and transformation of As in the environment are governed by geochemical as well as biological processes, generating an As biogeochemical cycle (Wang and Mulligan, 2006).

The marine environment plays an important role in the biogeochemical cycle of metals and metalloids since it covers 71% of the earth surface. In seawater, the average concentration of As is about 0.035 μM (Mukhopadhyay et al., 2002) and occurs mainly as inorganic As, in both the trivalent (As(III)) and pentavalent (As(V)) state (Morita and Shibata, 1990). Small quantities of organic As species, such as monomethylarsonate (MMA) and dimethylarsenate (DMA), were also found in seawater, particularly under conditions of increased biological activity (Francesconi, 2005). Algae are widely distributed in oxygenated seawater, where As occurs as a toxin mainly in the pentavalent (As(V)) form (Francesconi, 2005). Although the average concentration of As in seawater is typically low (Mukhopadhyay et al., 2002), as an analog of phosphate, As(V) absorption by marine algae is enhanced along the uptake patterns of phosphate which occurs at low levels (0.065–0.113 μM) in marine environments (Sanders, 1980; Takahashi et al., 1990; Rahman et al., 2008). Marine algae have the ability of accumulate As 3–4 orders of magnitude higher than that in seawater, yielding less toxic or non-toxic organic arsenic compounds (Edmonds and Francesconi, 1981; Šlejkovec et al., 2006). Many studies on As speciation in marine organisms have established that arsenobetaine (AsB) is the major As species found in animal tissues, while in plants like algae, arsenosugars are the most frequently occurring As species (Murray et al., 2003).

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Marine microalgae have been the subject of many As metabolic studies because of their ecological and nutritional importance. Much of what we know about As detoxification in marine microalgae derived from studies focusing on As methylation and arsenosugar formation. For example, unicellular microalgae grown in radiolabeled (^{74}As) As(V) were reported to produce As(III), MMA and DMA (Andreae and Klumpp, 1979). Edmonds et al. (1997) reported that when the unicellular microalga, *Chaetoceros concavicornis* was grown axenically in As(V) enriched water, oxo-arsenosugar-sulfate (arsenosugar 4) was identified as the major As metabolite. Cullen et al. (1994) cultured a unicellular microalga, *Polyphysa peniculus*, in artificial seawater with As(V) and As(III), and identified DMA as the major metabolic product in both the cells and the medium. However, little is known about whether marine microalgae can volatilize As and their potential contribution to the biogeochemical cycle of As.

Arsenic biovolatilization has first been recognized in a number of fungi in the late 1800s. The product, a gas with garlic odor remained unidentified until 1933 when Challenger et al. (1933) established its identity as trimethylarsine (TMAs). It has since been reported that organisms can volatilize inorganic As to arsines (arsine AsH_3, mono-, di- and trimethylarsines, $MeAsH_2$, Me_2AsH, and TMAs, respectively), and contribute to the As biogeochemical cycle (Mestrot et al., 2011). Recently, some studies reported As volatilization from soil, geothermal environments and freshwater into the atmosphere by bacteria, thermoacidophilic eukaryotic microalga and fresh water microbes. Qin et al. (2006, 2009) showed that volatile trimethylarsine (TMAs) is the final product of the methylation pathway both in bacteria, as in *Rhodopseudomonas palustris*, and in the thermoacidophilic eukaryotic microalga, *Cyanidioschyzon* sp. isolate 5508. Similarly, arsenic methylation and volatilization were also reported in three freshwater cyanobacteria, significantly contributing to As biogeochemical cycling (Yin et al., 2011). Currently, studies on As biovolatilization in marine environment are very scarce. Although not well studied yet, biological production of volatile As compounds in marine environments is considered to be an important part in the global As biogeochemical cycling (Michalke and Hensel, 2004).

Ostreococcus tauri is ubiquitous and was isolated in 1994 as the smallest free-living eukaryotic organism. As a model organism of natural marine phytoplankton assemblage with cosmopolitan distribution from coastal to oligotrophic waters, its genome was sequenced in 2006 (Derelle et al., 2006). However, few studies of this marine microalga on metal metabolism have been carried out, and whether it is able to tolerate and accumulate high levels of arsenic as many marine microorganisms do remains unknown. Even less is known about the ability of these microalgae for As biotransformation and biovolatilization. In this manuscript, we report As transformation and volatilization by this marine unicellular microalgae, suggesting the potentially significant contribution made by marine phytoplankton to the global biogeochemical cycling of As.

2. Materials and methods

2.1. Growth conditions for O. tauri

O. tauri was obtained from the Roscoff Culture Collection, France and was grown in 100 mL of Keller medium in Artificial Seawater (KASW) (Keller et al., 1987) in 250 mL conical flasks. Experiments were carried out in a controlled-environment growth chamber under the following conditions: 16-h light period/day with a light intensity of approximately 280 μmol m^{-2} s^{-1}, 22/20 °C of day/night temperatures, and 60% relative humidity. The inoculum used in the experiments came from an exponential phase culture.

2.2. Growth of microalgae exposed to As

Arsenic was supplied as $NaAsO_2$ and Na_3AsO_4 at indicated concentrations (10, 30, 50, 100, 500 μM) to investigate As tolerance and accumulation. The As concentrations were selected for potentially contaminated environments, not to represent the ambient pristine coastal environment. *O. tauri* (10 mL at the exponential growth stage) was incubated in 90 mL KASW. The experiment was conducted in triplicate. Aliquots of 2 mL medium were taken at 0, 2, 4, 6, 8, 10, 12, 14, 16 d after addition of As, and growth was measured as optical density at 600 nm (OD_{600}). Growth rates determined by optical density measurements at day 16 *versus* the control were used as biological end points to estimate the effect of As species on *O. tauri*. Results were fit with a dose–response curve equation, incorporated in the OriginPro 8 program, and EC50, the concentration corresponding to 50% inhibition of the growth rate was evaluated.

2.3. Cellular As speciation after exposure to inorganic arsenic

After exposure to 30 μM As(III) or As(V) for 4, 8, 12, or 16 d, microalgae were collected by centrifugation (6000 rpm), and subsequently rinsed with deionized water and ice-cold phosphate buffer (1 mM K_2HPO_4, 5 mM MES and 0.5 mM $Ca(NO_3)_2$) for 10 min to remove apoplastic As. Microalgal cells were oven-dried at 70 °C and kept in 50 mL polypropylene tubes with 10 mL of 1% HNO_3 overnight. The samples were extracted with microwave assistance (CEM Microwave Technology Ltd., Matthews, NC, USA). The working program was as follows: 55 °C for 10 min, 75 °C for 10 min, and 95 °C for 30 min, with 5 min ramp time between each stage (Zhu et al., 2008; Yin et al., 2011). The supernatants were filtered through 0.45 μm filters and kept in −20 °C before analysis. The speciation of As in microalgal cells was determined by HPLC–ICP–MS (7500a; Agilent Technologies) as described in Section 2.7. In order to determine whether the As biotransformation are results of adsorption to dead cells or enzymatic activity of live microalgal cells, the experiments with dead cells (medium incubated with *O. tauri* was sterilized at 121 °C for 20 min) were carried out as the control treatment following the same procedure as described above.

For arsenosugar analysis, microalgae were exposed to 10 or 30 μM As(V) for 4 weeks, and collected in the same way as described above. Microalgal cells were freeze-dried and extracted in 2 mL deionized water following a previously developed microwave-assisted extraction method (García-Salgado et al., 2011). The extraction process was: 90 °C for 5 min, and repeated two times. The extracts were centrifuged at 4 °C (6000 rpm) for 10 min, the two supernatants were mixed and filtered through a 0.45 μm filter and kept in −20 °C before further analysis.

2.4. Arsenic transformation by cells and cell-free supernatant

O. tauri was cultivated for 2 weeks, after which the medium and microalgal cells were separated by centrifugation (6000 rpm) under sterile condition. The supernatant was filtered by a sterilized 0.22 μM nylon filter. Microalgal cells were transferred to the new sterile fresh medium. Arsenate (1.6 μM) was added to the supernatant and medium in triplicates each. Aliquots of 2 mL medium were taken at 0, 10, 20, 40, 60, 90 h and filtered through a 0.45 μM nylon filter and kept in the refrigerator at −20 °C until further analysis.

2.5. Arsenic biomethylation by O. tauri

O. tauri was exposed to 30 μM As(V) during a time-course experiment to investigate the process of As biomethylation by *O.*

tauri. The experiment was conducted in triplicate. Aliquots of 2 mL medium were taken at 3, 6, 8, 10, 12, 14, 16 d after addition of As(V). The medium was filtered with 0.45 μM nylon filter, and kept in −20 °C before As speciation analysis. The control treatment (without microalgal cells) was carried out in the same way as described above.

2.6. Chemotrapping of volatile As

Volatilized As by *O. tauri* was trapped as reported (Mestrot et al., 2009). The trap tube was as follows: silica gel (0.5-mm diameter) was kept in 5% HNO_3 overnight, washed with Millipore water, impregnated with 10% $AgNO_3$ solution (w/v) overnight, and dried in the oven at 70 °C. *O. tauri* was cultured in a vessel designed as a volatile As trap consisting of a triangular flask (250 mL) containing 100 mL culture medium with 20 μM As(III) or As(V) and a glass joint with inlet and outlet of gas. The trap tube was connected to the outlet, and a super silent adjustable air pump (ACO-9601; 2-W power) was linked to the inlet to supply carbon dioxide for the growth of *O. tauri*. After 4 weeks culture, the trapped As on the silica gel was eluted in 1% HNO_3 using a microwave digestion system. The supernatant were filtered by a 0.45 μm filter and total As was analyzed by ICP–MS.

2.7. Arsenic speciation analysis

Arsenic speciation was determined by HPLC–ICP–MS (7500a; Agilent Technologies) as described (Zhu et al., 2008). Chromatographic columns were obtained from Hamilton and consisted of a precolumn (11.2 mm, 12–20 μm) and a PRP-X100 10-μm anion-exchange column (250 × 4.1 mm). The mobile phase was consisted of 10 mM diammonium hydrogenphosphate ($(NH_4)_2HPO_4$) and 10 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using nitric acid. The mobile phase was pumped through the column isocratically at 1 mL min^{-1}. Arsenic species in the samples were identified by comparing their retention time with those of the standards, including arsenite (As(III)), arsenate (As(V)), dimethylarsenate (DMA), monomethylarsonate (MMA), and quantified by external calibration curves with peak areas.

For the detection of arsenosugars, the mobile phase consisting of 30 mM ammonium dihydric phosphate ($NH_4H_2PO_4$) was adjusted to pH 5.6 with aqueous NH_3. The mobile phase was pumped through the column isocratically at 1.5 mL min^{-1}. The As standards included oxo-arsenosugar-glycerol (arsenosugar 1), oxo-arsenosugar-phosphate (arsenosugar 2), oxo-arsenosugar-sulfonate (arsenosugar 3) and oxo-arsenosugar-sulfate (arsenosugar 4).

3. Results

3.1. Arsenic tolerance for O. tauri

Growth of the microalga *O. tauri* was not inhibited by As(III) or As(V) concentrations of up to 50 μM, and the lag phase was approximately 8 d. When As(III) and As(V) concentrations were at >100 μM, microalgal growth was markedly inhibited with the lag phase increased to 10 d (Fig. 1). Interestingly, during the time span of 2–8 d, when exposed to 10 and 30 μM As(III) or As(V), the growth rate was 39–57% higher than that in arsenic-free medium, indicating that microalgal growth was stimulated by low concentrations of As due to hormesis. To evaluate the toxicity of different inorganic As species on *O. tauri*, the effect of increasing concentrations of As(III) and As(V) on microalgal growth-rate inhibition after 16th days was studied. The dose–response data could be described satisfactorily using the log–logistic equation with an R^2 value of 0.964 for both As(III) and As(V). Based on the fitted

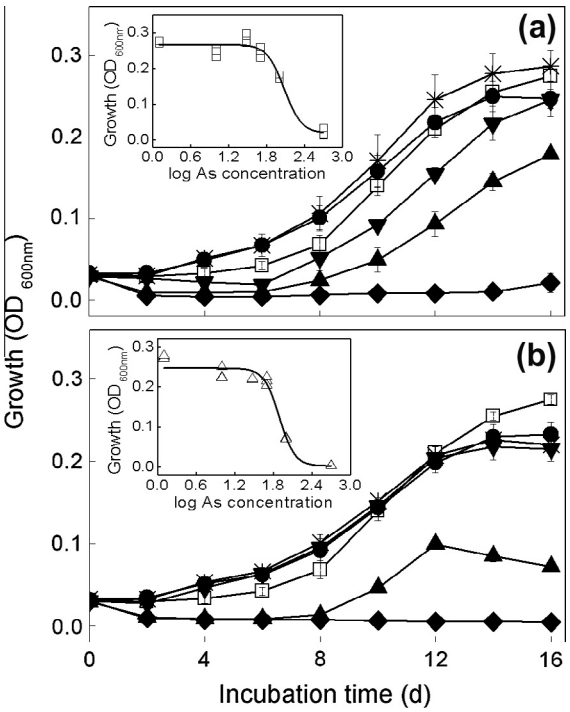

Fig. 1. Growth curves of *Ostreococcus tauri* in KASW containing different concentrations of arsenate (a) or arsenite (b): 0 μM (control), □; 10 μM, ●; 30 μM, ∗; 50 μM, ▼; 100 μM, ▲; 500 μM, ♦. 16 d arsenite or arsenate exposure effects on growth of *O. tauri* are evaluated. Lines are the fitted log–logistic curves. Data are mean ± standard deviation ($n = 3$). To allow log transformation, a small value (0.1) was added to the zero As concentration in the control treatment.

equations, the concentration of As(III) and As(V) in the medium that caused a 50% inhibition on growth (EC_{50}) were 78 and 120 μM respectively, indicating that this marine microalgae was more sensitive to As(III) than As(V) (Fig. 1).

3.2. Arsenite methylation by O. tauri

Arsenic speciation in cells was determined after exposure to 30 μM As(III) and As(V) (Fig. 2). When incubated for 4 d, two inorganic arsenicals (As(III) and As(V)), were detected as the only As species in cells. After incubation for 8 d with As(III) or As(V), organic arsenic, i.e. DMA was detected in microalgal cells, representing 2% and 6% of the total arsenic, respectively. Nevertheless, the percentage of DMA in microalgal cells decreased with a concomitant increase of incubation time. After 16 d growth, only 1% and 2% of the total As were DMA respectively. During the entire incubation time and whether exposed to As(III) or As(V), As(V) was always the major intracellular species, accounting for 76–93% of the total As associated with cells. The other inorganic form, As(III), was only a minor species, representing 5–24% of the total As. When exposed to As(III), the percentage of As(V) in the cell increased with incubation time, from 76% detected on day 8 to 86% on day 16. In medium with dead cells, no As biomethylation could be detected.

Cellular As concentration increased significantly ($P < 0.01$) with an increasing concentration of As(V) in the medium (0–50 μM) and a linear relationship was observed between cellular and medium As concentration (Fig. 3).

3.3. Various organic As products generated by microalgal cells of O. tauri

Cells and cell-free supernatants were prepared to investigate whether the microalgal cells or microalgal secretions contribute

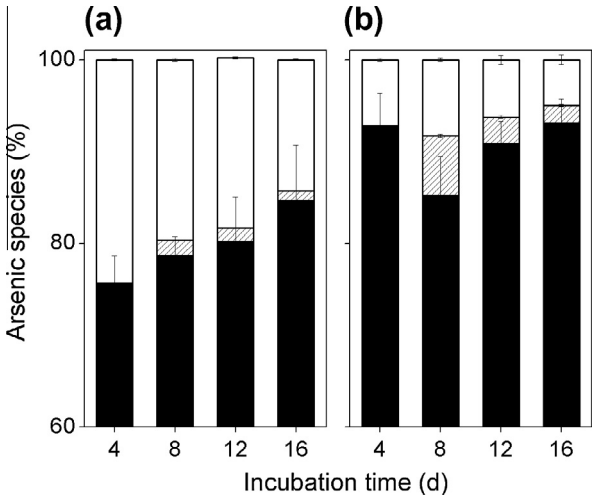

Fig. 2. The percentage of As species (As(V), closed bars; DMA, hatched bars; As(III), open bars) to cellular As accumulation (DW) in *O. tauri* after exposure to 30 μM arsenite (a) or arsenate (b) during a 16-d time course. Arsenic species in microalgal cells were analyzed on 4, 8, 12, 16 d. Data are mean ± standard deviation (n = 3).

to As biotransformation. When *O. tauri* was exposed to As(V) for 20 h, the concentration of As(V) declined, concomitant with an increase in As(III) levels (Fig. 4). In the medium with microalgal cells, about 28.1% and 42.7% of the As(V) was reduced to As(III) after 10 and 20 h respectively. When exposed to As(V) for 40 h, the percentage of As(III) declined to 24.7%, and after 90 h of incubation, only 9.3% of As(III) was detected in the medium with microalgal cells. Since the medium could hardly have had any effect in such a short period, the decline of As(III) concentration in the medium was possibly a result of As(III) sequestration into the vacuole by microalgal cells or re-oxidation of As(III) to As(V) in the medium. In the cell-free supernatant As(III) was detected at only 7.1%, and the percentage of As(III) to total As was stable during the incubation time. The reduction of As(V) to As(III) and the appearance of As(III) in the medium were directly mediated by microalgal cells, as cultural conditions and microalgal secretions (the supernatant) hardly had any effect on As transformations during the period of our experiment. No organic As was detected in the system possibly due to the short period of incubation (90 h). There was no organic As after 3 d incubation, but DMA was detectable after 6 d exposure (Fig. 5).

The total sum of As species remained stable during the experiment (16 d), suggesting proper mass balance and good quality control for As speciation analysis. Organic arsenic, i.e. DMA was

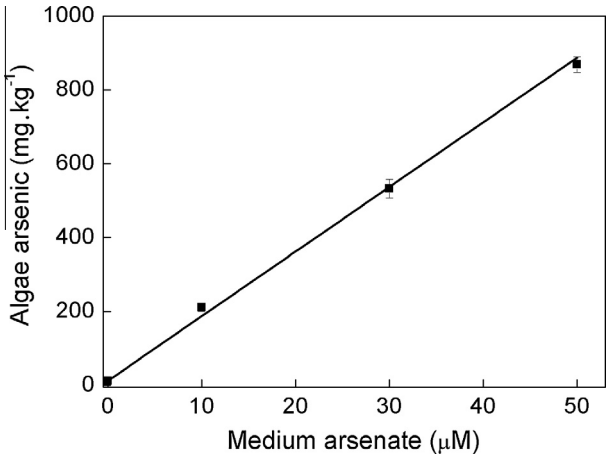

Fig. 3. Sum of the concentration of different As species (■) in *O. tauri* after exposure to 10, 30, 50 μM arsenate for 16 d. Data are mean ± standard deviation (n = 3).

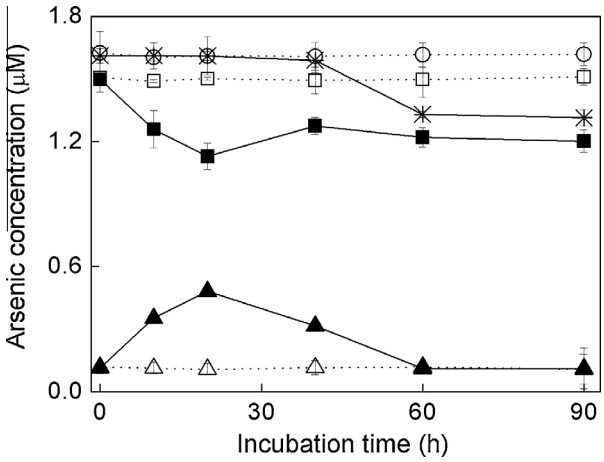

Fig. 4. Arsenic species in medium with *O. tauri* (As(III), ▲; As(V), ■; sum of different As species, ∗) or without *O. tauri* (control) (As(III), △; As(V), □; sum of different As species, ○) exposed to 1.6 μM arsenate during a 90-h time course. Arsenic species in medium were analyzed on 0, 10, 20, 40, 60, 90 h. Data are mean ± standard deviation (n = 3).

detected after incubation for 6 d in the medium with fresh microalgal cells (Fig. 5). During the first 14 d, DMA concentration increased over the incubation time, reaching 0.8 μM as the maximum concentration, accounting for 2.3% of the total As in the medium. By contrast, in the control (without microalgal cells), DMA was not detected during the entire incubation period. The percentage of As(III) was steady at about 0.7%, much less than the value with fresh microalgal cells (1.1%). No MMA was observed during the experiment.

When *O. tauri* was exposed to 10 or 30 μM As(V) for a long time-term (4 weeks), not only As(III) and DMA were detected, but also a small amount of oxo-arsenosugar-phosphate (arsenosugar 2) was detected with 1.7 ± 0.1 and 1.2 ± 0.03 mg kg^{-1} (DW) in microalgal cells respectively (Table S1). No DMA or oxo-arsenosugar-phosphate (arsenosugar 2) was detected in microalgal cells cultured without As addition.

3.4. Volatile As produced by O. tauri

When *O. tauri* was incubated with 20 μM As(III) or As(V) for 4 weeks, about 16.7 ± 0.7 and 3.1 ± 0.7 ng of volatile arsines were

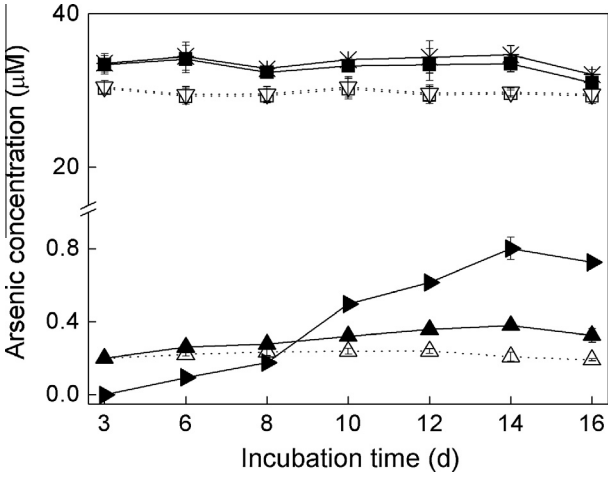

Fig. 5. Arsenic species in medium with *O. tauri* (As(III), ▲; As(V), ■; DMA, ; sum of different As species, ∗) or without *O. tauri* (control) (As(III), △; As(V), □; sum of different As species, ▽) exposed to 30 μM arsenate during a 16-d cultivation. Arsenic species were analyzed on 3, 6, 8, 10, 12, 14, 16 d. Data are mean ± standard deviation (n = 3).

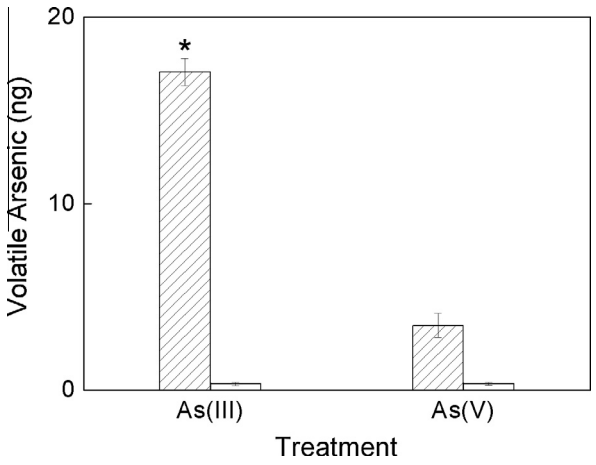

Fig. 6. Volatile As levels (ng) (with (hatched bars) or without (opened bars) *O. tauri* (control)) after exposure to 20 μM arsenite or arsenate for 4 weeks. Data are mean ± standard deviation ($n = 3$). The amount of volatile As generated by *O. tauri* under As(III) treatment was significantly ($P < 0.001$) different from that of As(V) treatment.

trapped respectively (Fig. 6). By comparison, only tiny amounts of volatile As (0.35 ± 0.08 and 0.35 ± 0.07 ng) were detected in the corresponding control (without microalgal cells) with 20 μM As(III) or As(V). This demonstrated that microalga *O. tauri* has the ability to volatilize As. The results of variance analysis using independent-samples *T* test showed that the amount of volatile As generated in the presence of As(III) treatment was significantly ($P < 0.001$) higher (by five times) than in the presence of As(V) treatment.

4. Discussion

The results described herein clearly demonstrated that the marine green microalgae *O. tauri* has the ability to methylate and volatilize As in seawater. To the best of our knowledge, this is the first report showing that marine microalgae have the capacity to volatize As. This is possibly due to the adaption of marine microalgae to its low phosphate growth environment, which causes high As absorption, and subsequent metabolic mechanisms to detoxify absorbed As from the environment through methylation and volatilization.

The current results confirm that this marine microalga *O. tauri* has high tolerance towards As. When exposed to 10 and 30 μM As(III) or As(V), microalgal growth was stimulated by low concentrations of As during the time span of 2–8 d, possibly due to hormesis. Similar growth stimulation by As had also been reported when the green microalga *Chlorella* sp. isolated from an As-contaminated lake was exposed to 1 μM As(V) or *Chlorella vulgaris* was exposed to 133–266 μM As(III) (Suhendrayatna et al., 1999; Knauer and Hemond, 2000). The EC50 values of As(III) and As(V) for *O. tauri* were 78 and 120 μM respectively, indicating that this marine microalga is more sensitive to As(III) than As(V) in Keller medium in Artificial Seawater (KASW). This observation is in good agreement with results showing that As(III) is more toxic than As(V) to marine microalgae (Yamaoka et al., 1999). Although *O. tauri* grew better in medium containing 50 μM As(III) than 50 μM As(V) on day 4–12, after incubation for 14 d, the growth of *O. tauri* was much more inhibited in medium with 50 μM As(III) than with 50 μM As(V), indicating that As(III) is more toxic than As(V) for *O. tauri*. Considering the concentrations of 50 μM As(III) and As(V) are lower than EC50 for As(III) and As(V), respectively, the inhibition of 50 μM As on microalgal growth might not be significant. Therefore, the better growth of *O. tauri* in medium containing 50 μM As(III) might reflect small variations in growth. *O. tauri* was able to tolerate both As(III) and As(V) up to 100 μM, much higher than the average As concentration in seawater. Possibly, this is due to the high phosphate concentration of 6 μM in KASW, which is higher than the low level phosphate concentrations (0.065–0.113 μM) occuring in the marine environments (Sanders, 1980). Karadjova et al. (2008) have reported that after the enrichment of seawater with phosphate (from 0.065 to 41.94 μM), the EC50 for As(III) and As(V) for the green microalga *Chlorella salina* in seawater, increased from 63 nM to 13 μM and 31 nM to 16 μM respectively, it is reasonable to speculate that in KASW the As toxicity was decreased by the high phosphate concentrations (60 μM) which was similar to the results that the increase in phosphate in the growth medium decreased the toxicity of As(V) for the freshwater microalgae *Monoraphidium arcuatum* as reported (Levy et al., 2005).

The process of As biomethylation by this unicellular marine microalgae may have followed a serial procedure, so as to detoxify the high amounts of accumulated As in microalgal cells. Microalgal cells might first take up As(V), the dominant form of As in oxygenated seawater (Francesconi, 2005), probably via the phosphate transport system (Rosen and Liu, 2009). After exposure to As(V) for 10, 20, 40 h, As(III) was detected in the medium which was possibly generated through As(V) reduction, and then extruded into the medium by microalgal cells in order to detoxify. With increasing incubation time, As(III) in microalgal cells could stepwise be methylated mainly to DMA, the main organic As species detected in microalgal cells (Fig. 2), and then some of the DMA might be excreted into the medium as indicated in Fig. 5. Similar As transformation processes have been reported in the unicellular eukaryotic red microalga *Cyanidioschyzon merolae*, which takes up As(V) originating from As(III) oxidation, and then reduces As(V) to As(III) in the cytoplasm for subsequent efflux or methylation (Qin et al., 2009).

The organic As species, such as MMA and DMA were found in seawater, particularly under conditions of increased biological activity (Francesconi, 2005), which suggests the ability of marine organisms to produce and extrude methylated As compounds. However, MMA which has been predicted as an intermediate product of As biomethylation as suggested by Challenger (1945) was not detected in our experiment. We speculated that MMA was not present due to the speed of MMA transformation to DMA. Similar result has also been reported by Cullen et al. (1994), when *P. peniculus* was treated with 12 μM As(V), DMA was the organic As specie and no MMA was found in cells. A new metabolic pathway for As methylation has recently been proposed (Naranmandura et al., 2006), it speculated that the As methylation reaction takes place with simultaneous reductive rather than stepwise oxidative methylation. In this model, As(III) is directly methylated to DMAs(III) through the process: As(III)–MMAs(III)–DMAs(III), and then oxidized to the pentavalent methylated As metabolites which were suggested as the end product of metabolism, rather than intermediates.

In addition to As(III) efflux and biomethylation, organisms have evolved many other As(III) detoxification mechanisms. As(III) can also be pumped into the vacuole for sequestration by eukaryotics, such as yeast and the fern *Pteris vittata* (Bhattacharjee and Rosen, 2007; Indriolo et al., 2010). It is possible that *O. tauri*, a eukaryotic microalga, might also sequester As(III) in the vacuole as an additional detoxification pathway. As(III) could also be chelated by phytochelatins (PCs) as other studies have revealed that in plants, algae and some fungi, phytochelatins (PCs) are involved in detoxification and homeostasis of trace metals (Grill et al., 1985). It has been reported that in marine diatom *Phaeodactylum tricornutum*, PCs were produced in response to copper and cadmium exposure,

as the mechanism for detoxification (Morelli and Pratesi, 1997), it is therefore reasonable to assume that PCs are also involved in As detoxification by *O. tauri*.

In addition, the ability of marine microalgae for arsenic biovolatilization was first confirmed in this study. Volatile As was detected after incubation of *O. tauri* for an extended period of time (4 weeks) with As(III) or As(V). Similar As biovolatilization abilities have been reported in other organisms. For some bacteria, fungi and freshwater microalgae, volatile As compounds, namely AsH_3, $MeAsH_2$, Me_2AsH, and TMAs, have been reported to be transformed from inorganic As, and As methylation was identified as the main pathway for As volatilization (Qin et al., 2006, 2009; Mestrot et al., 2011; Yin et al., 2011; Urík et al., 2007). Mestrot et al. (2011) confirmed that TMAs, the dominant end product of inorganic As methylation, was the main volatized As species produced by soils in microcosms experiments. Moreover, TMAs as the final and volatile end product of the methylation pathway has also been reported in three freshwater cyanobacteria (Yin et al., 2011). Furthermore, it was demonstrated that As biomethylation and biovolatilization in this marine unicellular eukaryotic microalga differed between treatments with As(III) and As(V). When we examined the amount of volatile As generated undergoing the treatment with the same concentration (20 μM) of As(III) or As(V), volatile As generated was about five times more under As(III) treatment than under As(V) treatment (Fig. 6). When the microalgae were exposed to As(V), which is the predominant As species in seawater, As(V) has to first be reduced to As(III), and then methylation can occur in a series of steps. In this case, As(V) reduction might be the rate-limiting factor for As methylation and volatilization by *O. tauri*. As a consequence, when treated with As(III), arsenic methylation could take place in microalgal cells more quickly, and the amount of volatile As which was generated mainly through As methylation was significantly higher.

The ability of transforming As(V) to arsenosugars for *O. tauri* has been shown in this study. Small amount of oxo-arsenosugar-phosphate (arsenosugar 2) was detected in microalgal cells after exposure to 10, 30 μM As(V) for 4 weeks. A similar study of the unicellular microalga *Chlamydomonas reinhardtii* reported that it could rapidly transform As(V) into oxo-arsenosugar-glycerol (arsenosugar 1) after not more than 10 min, and after 3 h oxo-arsenosugar-phosphate (arsenosugar 2) was detected at low concentrations (Miyashita et al., 2011). The production of arsenosugars has previously been suggested to be a subsequent step after As(III) methylation. Here, DMA(III) was oxidized by the addition of the adenosyl group from SAM (S-adenosylmethyltransferase), and this nucleoside could be used to produce a range of arsenosugars through glycosidation (Murray et al., 2003).

Considering the huge surface area of the ocean on earth (71%) as well as the abundance of marine species in marine environments, and the now confirmed As biomethylation and biovolatilization ability of many marine organisms, marine environments could play an important role in As biogeochemical cycling. The significant role that the marine environment plays in the As geochemical cycle has already been highlighted. Lantzy and Mackenzie (1979) proposed significant fluxes of As from the sea surface to the atmosphere. Chilvers and Peterson (1987) concluded that an emission factor of 0.053 μg As gm^{-1} sea-salt spray could give a global emission of 26.5 tonnes $year^{-1}$. Moreover, based on our results, marine microalga may also volatize As from the seawater and thus contribute to the global As emissions, especially in As contaminated coastal environments. Since *O. tauri* has been recognized as a model organism of natural marine phytoplankton assemblage, we speculate the contribution made by marine phytoplankton biovolatilization to As biogeochemical cycling would be significant as well. The As volatilization ability of *O. tauri* revealed in this study may have implications for understanding the role of marine microalga in As biogeochemical cycle.

5. Conclusions

Our study demonstrated that the marine microalga *O. tauri* can transform inorganic As into the organic As species DMA, oxo-arsenosugar-phosphate (arsenosugar 2), and volatilize As, confirming its capability of As biomethylation and biovolatilization. As(V) reduction was speculated to be the rate-limiting factor for As methylation and volatilization by *O. tauri*. Moreover, taking into consideration of the huge surface area of the ocean on earth (71%) and the vast number of oceanic phytoplankton, marine phytoplankton may play a significant role in the biogeochemical cycling of As.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2013.04.063.

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