

Arsenic biotransformation by cyanobacteria from mining areas: evidences from culture experiments

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Abstract Elucidating the role of cyanobacteria in the biotransformation of arsenic (As) oxyanions is crucial to understand the biogeochemical cycle of this element and indicate species with potential for its bioremediation. In this study, we determined the EC₅₀ for As(III) and As(V) and evaluated the biotransformation of As by *Synechococcus* sp. through high-performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and X-ray absorption fine structure spectroscopy (XAFS). *Synechococcus* sp. exhibited higher sensitivity to As(III) with an EC_{50, 96 h} of 6.64 mg L⁻¹ that was approximately 400-fold lower than that for As(V). Even though the cells were exposed to concentrations of As(III) (6 mg L⁻¹) approximately 67-fold lower than those of As(V) (400 mg L⁻¹), similar intracellular concentrations of As (60.0 µg g⁻¹) were observed after 30 days. As(V) was the predominant intracellular As species followed by As(III). Furthermore, organic As species such as

monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were observed in higher proportions after exposure to As(III). The differential toxicity among As oxyanions indicates that determining the redox state of As in the environment is fundamental to estimate toxicity risks to aquatic organisms. *Synechococcus* sp. demonstrated potential for its application in bioremediation due to the high accumulation of As and production of As organic compounds notably after exposure to As(III).

Keywords Cyanobacteria · Arsenic speciation · *Synechococcus* · Growth inhibition · Bioremediation

Introduction

Contamination of soil and water by arsenic (As) is a global problem. Industrial activities such as mining or construction of tube wells in arseniferous geological deposits are known sources of environmental As. Typical As concentrations in water are less than 10 µg L⁻¹ (Smedley and Kinniburgh 2002); however, higher levels have often been found in groundwater. The biogeochemistry of As is a complex system and involves transformation into various chemical species with different levels of toxicity. Under aerobic conditions, arsenate (H₂AsO₄⁻/HAsO₄⁻²) is expected to be the predominant species, while under reducing conditions, arsenite [As(OH)₃] is the anticipated major species. However, microbial activity has a strong influence on As availability and speciation. In lakes, occurrences of As(III) predominance have been recorded in the epilimnion and attributed to the absorption, excretion, and reduction of As by phytoplankton blooms (Hellweger and Lall 2004; Kuhn and Sigg 1993). Thus, concentrations of both As(III) and As(V) may become available even in aerobic environments.

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Toxic elements are persistent, and processes involving their conversion into nonbioavailable residues or their biotransformation into species less harmful to the environment have been already reported (Valls and de Lorenzo 2002). The possibility of using microorganisms in clean technologies for environmental decontamination has spurred the search for resistant organisms that are capable of biotransforming toxic elements such as As. Cyanobacteria are of interest because of their dominant position in aquatic environments impacted by different types of pollutants (Su et al. 2012; Garcia-Meza et al. 2005). Moreover, several studies have reported their ability to biotransform oxyanions of As into organic forms (e.g., Guo et al. 2011; Yin et al. 2011).

The detoxification mechanisms of arsenate [As(V)] by microorganisms include absorption, reduction, and efflux in arsenite [As(III)] or methylated forms (MMA/DMA) (Rosen 2002). Other compounds such as arsenosugars are also produced by cyanobacteria (Miyashita et al. 2012). The first step in the pathway of As biotransformation is its reduction to As(III) by arsenate reductase (Pandey et al. 2013; Li et al. 2003). Subsequently, a methyltransferase catalyzes the transfer of the methyl group of S-adenosylmethionine (SAM) to As(III) (Shen et al. 2013). In both eukaryotic and prokaryotic organisms, methylation operates as a detoxification mechanism that produces less toxic As chemical species. Immobilization of As in the inner of cyanobacterial cells is also considered a detoxification mechanism (Ybarra and Webb 1998). Molecules containing cysteine residues such as the tripeptide glutathione (γ -glutamylcysteinylglycine, GSH) are critical in this process due to the high As(III) affinity for sulfhydryl groups (Shen et al. 2013). Thus, by using some detoxification pathways, cyanobacteria plays an important role on the dynamics of As in the environment.

Arsenic metabolism by cyanobacteria has mainly been studied in water-bloom-forming species from eutrophic lakes (Huang et al. 2014; Wang et al. 2013; Guo et al. 2011). Microorganisms have undergone periods of exposure to toxic elements throughout evolution and thus have evolved mechanisms to coordinate element uptake and the expression of particular detoxification genes (Silver 1998). The recent anthropogenic mobilization of metals has resulted in the creation of new metal-enriched niches with a high selective pressure for resistant organisms (Valls and de Lorenzo 2002). Based on the hypothesis that cyanobacterial strains obtained from contaminated environments are more likely to possess efficient mechanisms of As biotransformation, in this study, we analyzed the As species formed by *Synechococcus* sp., a nonbloom-forming cyanobacterial strain obtained from a stream in a mining area contaminated with As.

Materials and methods

Obtaining the cyanobacteria culture

The strain was obtained from a stream located downstream of a gold processing plant in the municipality of Nova Lima (19° 58' 74.8" S, 43° 49' 25.9" W) in Minas Gerais state, Brazil. Micropipetting and plating were used to obtain monospecific algal cultures according to Allen (1973). The strain is currently maintained in the algae culture bank of the Laboratory of Limnology, Ecotoxicology and Aquatic Ecology–LIMNEA of the Institute of Biological Sciences of Universidade Federal de Minas Gerais. Taxonomic characteristics were consistent with taxonomic description for the morphogenus *Synechococcus* C.Nägeli, 1849, according to Komárek and Anagnostidis (1998). Cells are isolated and rarely form short pseudofilaments; they are cylindrical and straight, without mucilage, 1.5–2 μm long, 1–1.3 μm wide, and without aerotopes (Sant'Anna et al. 2007; Komárek and Anagnostidis 1998).

Cyanobacteria taxonomy is going through a transition period, where many species are being reviewed on a polyphasic approach that relies on molecular, ultrastructural, morphological and ecological data, see Komárek et al. (2014). *Synechococcus* is a broad genus that is poorly morphologically and ecologically defined; furthermore, it belong to the simplest cyanoprokaryotic organisms in relation to morphology, living as solitary, oval to cylindrical cells (Komárek et al. 1999, 2014).

The 16S rRNA sequence, from the present *Synechococcus* sp. strain, was obtained. The identity of our strain was $\leq 93.0\%$ when compared with those from *Synechococcus elongatus* PCC6301 (type-strains of this genus). Recently, many strains of *Synechococcus* have been sequenced and phylogenetic analysis already resulted in the separation of 12 clusters, but their differences did not always reach 95 % of similarity (Komárek 2010). The family Synechococcaceae is in great need of study and revision at all taxonomic levels (Komárek et al. 2014).

Phylogenetic studies have shown a big diversity of cyanobacteria isolated from Brazilian environments, such as mangroves (Silva et al. 2014; Genuario et al. 2015), saline-alkaline lakes (Vaz et al. 2015), and subaerophytic environments (Aguiar et al. 2008), and new taxa were isolated and identified by ultrastructural, ecological, and molecular analysis.

Growth inhibition

Acute toxicity tests were conducted as recommended by the 201 OECD (2006) protocol. Cultures were grown in Erlenmeyer flasks (150 mL) containing BG-11 medium supplemented with 750 mg L^{-1} 3-(N-morpholino) propane sulfonic

acid (MOPS) buffer (pH=7), kept under constant agitation and controlled conditions of light ($27 \mu\text{mol cm}^{-2}$) and temperature ($20 \pm 1 \text{ }^\circ\text{C}$). In the exponential growth phase (3 days), the cultures containing about 10^6 cells mL^{-1} were exposed to As(V) or As(III) solutions ($n=3$) from $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ and NaAsO_2 (Sigma-Aldrich), respectively, to obtain geometric series of concentrations. Two series were established based on the toxicity range determined from preliminary testing, i.e., 0, 500, 900, 1620, and 2916 mg L^{-1} [As(V)] and 0, 4.80, 5.52, 6.35, 7.29, and 8.38 mg L^{-1} [As(III)]. Cell growth was measured daily, based on optical density (OD) at 631 nm. The cell number was obtained by a linear correlation with the optical density of the control [$y=(25.82 \times -0.844) E7$, $R^2=0.96$]. Growth rates were calculated according to the equation $\mu_{i,j}=\ln X_j-\ln X_i/(t_j-t_i) \text{ day}^{-1}$, where μ is the average specific growth rate from time i to j in days, X_i is the number of cells mL^{-1} at time i , and X_j is the number of cells mL^{-1} at time j . The growth rates of each treatment were compared by one-way analysis of covariance (ANCOVA). Growth inhibition was obtained from the equation $\%I_r=[\mu_c-\mu_t/\mu_c] \times 100$, where $\%I_r$ is the percentage of inhibition for a specific growth rate, μ_c is the average growth rate in the control group and μ_t is the average growth rate in experimental replicates.

The As concentration in the supernatant of all replicates was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Concentrations were log-transformed, and the $\text{EC}_{50, 96 \text{ h}}$ was estimated by nonlinear regression using a four-parameter logistic equation restricted to the interval (0, 1). The standard error of the parameters was assessed by nonparametric bootstrapping and the 95 % confidence interval was calculated using the adjusted bootstrap percentile method (Nyholm et al. 1992; Efron and Tibshirani 1986). The analyses were performed using the software R (R Core Team 2014).

Speciation experiment

Triplicates of cyanobacteria cultures (1500 mL) were cultivated in BG-11 medium in Erlenmeyer flasks. For inoculation, the cultures were centrifuged and added to the media to yield a final concentration of 7×10^7 cells mL^{-1} . Freshly prepared solutions of sodium arsenate or sodium arsenite were added to obtain a final concentration of 400 mg L^{-1} As(V) or 6 mg L^{-1} As(III). Algae-free duplicates were prepared using the same conditions as a control to isolate the effect of cyanobacteria metabolism on As chemical species. All experimental samples were constantly aerated and mixed with filtered air ($0.22 \mu\text{m}$) under constant illumination from cool lamps.

Samples were collected at the beginning of the experiment and on 30th day. The culture was centrifuged and two phases were obtained: the biomass and the supernatant. To remove residual As, the biomass samples were washed three times with distilled water, lyophilized (L101, Liobras) and stored

at $-20 \text{ }^\circ\text{C}$. Samples of the supernatant and algae-free controls were stored at $4 \text{ }^\circ\text{C}$ until analysis. To determine the total As concentration, 25 mg of sample was mineralized by adding 3 mL of HNO_3 (96 %) and 1 mL of H_2O_2 (30 %), followed by digestion in a microwave (ETHOS One model, Milestone Microwave Systems, Shelton, USA) at $200 \text{ }^\circ\text{C}$ for 30 min at 45 bar. The final volume was adjusted to 25 mL (Truus et al. 2007). Determinations of As were performed using ICP-OES (Perkin Elmer Optima 4300 DV) and the mean of total As concentration in the supernatant was compared between groups by paired t test.

To extract As species from the biomass, 10 mL of an extraction solution, consisting of HNO_3 (2 %) and methanol (2 %), was added to 50 mg samples of the lyophilized biomass, followed by rotational agitation for 16 h, incubation in a water bath at $60 \text{ }^\circ\text{C}$ for 2.5 h and filtration with a cellulose filter ($0.20 \mu\text{m}$) (Batista et al. 2011). The supernatant samples were diluted in the same extraction solution and filtered. Speciation analyses were performed using high-performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) (Elan DRC II PerkinElmer, Norwalk, CT) with an anion exchange column (PRP X-100, $150 \times 4.6 \text{ mm}$, $5 \mu\text{m}$, Hamilton, Reno, NV, USA). The mobile phase consisted of 10 mM $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ (98 % v/v)+2 % (v/v) methanol, pH 8.5. The analyses were performed at the Laboratório de Toxicologia e Essencialidade de Metais, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo.

As coordination

X-ray absorption fine structure spectroscopy (XAFS) measurements were performed on the supernatant and lyophilized biomass of cultures exposed to As(V) at the Laboratório Nacional de Luz Síncrotron (LNLS/Campinas/Brazil). The standards used included As(V) and As(III) for As(V)-O and As(III)-O bonds, respectively, and DMA for the As-C bond. Samples were measured in the liquid phase [0.1 M] by filling a cell of 3 mm sealed with kapton tape or in solid phase (crystalline) by preparing a pastille containing 0.4 g of the As compound and 0.6 g of boron nitride. The As-S bond standard was freshly prepared by dissolving GSH and As(III) at a molar ratio of 1:4.

As K-edge (11,868 eV) data were collected at XAFS2 workstation operating with beam energy of 1.37 GeV and using a Si (111) double crystal monochromator. Samples were measured in transmission mode (standards and supernatant) at room temperature and fluorescence mode (biomass) at a temperature of 5 K. Energy calibration was monitored during data collection by acquiring a reference gold foil for the measurement of the As spectrum. The software Athena (Ravel and Newville 2005) was used for background subtraction, Fourier transform, and treatment of the data.

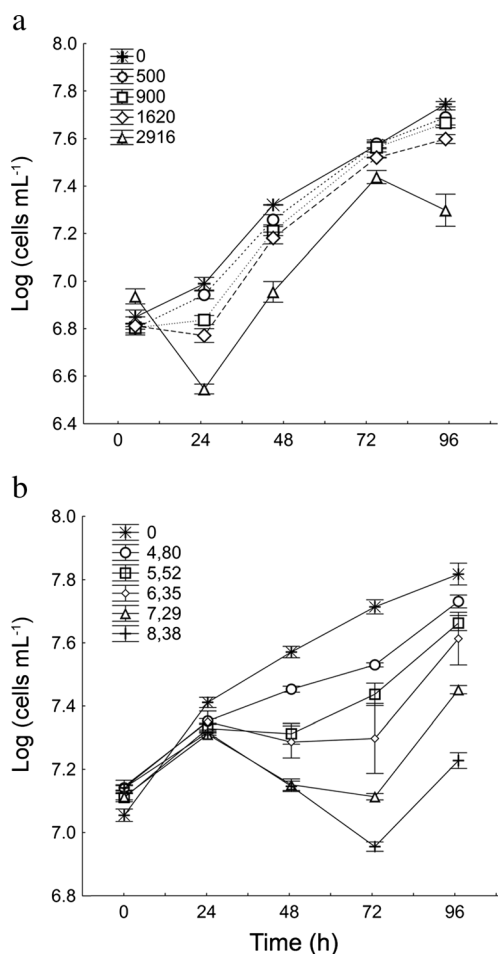


Fig. 1 Growth curves of *Synechococcus* sp. exposed to increasing concentrations (mg L⁻¹) of **a** As(V) or **b** As(III) for 96 h. Control group, $n=4$; experimental group, $n=3$, $p<0.05$, ANCOVA

Results

Growth inhibition

The cyanobacteria growth rate gradually decreased with the increasing of As concentrations ($p<0.05$) (Fig. 1). The As(V) concentration required to reduce significantly the growth was two orders of magnitude higher than the As(III), showing more sensitivity to As(III) ($p<0.05$). The concentration of As(V) that effectively reduced the growth rate by 50 % ($EC_{50, 96 h}$) was 2642.9 mg L⁻¹, with 95 % confidence interval (95 % CI) of 2372.7 to 2840.9, while the $EC_{50, 96 h}$ for As(III) was 6.64 mg L⁻¹, with a 95 % CI of 6.30 to 7.14 mg L⁻¹ (Fig. 2). The concentrations of As(III) or As(V) in the supernatant remained constant after 96 h.

As speciation

After exposure to As(V), the predominant intracellular As chemical species found was As(V), followed by As(III), with an expressive concentration (Fig. 3a). The first peak in the

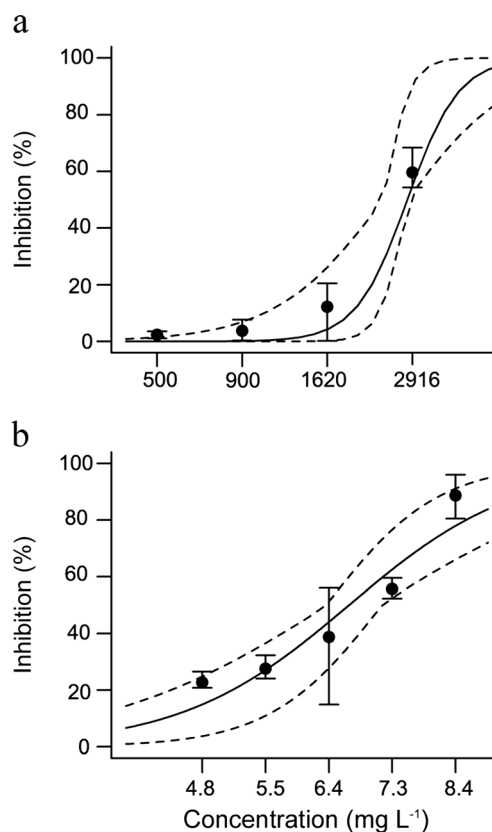


Fig. 2 Percentage growth inhibition of *Synechococcus* sp. exposed to As for 96 h. **a** As(V); **b** As(III)

chromatogram (Fig. 3a, b), named AsB, exhibited the same retention time as arsenobetaine. DMA and MMA were detected at low levels. The same intracellular As chemical species were observed after exposure to As(III) with a significant increase in the intensity of the signals for AsB (Fig. 3b). Other organic As species (MMA and unidentified species (UiS)) were also detected. Some variations on retention time were observed due to the high number of injections during the experiments and the complexity of the matrix. As expected, AsB exhibited the lowest retention time followed by As(III), DMA, MMA, and finally the most polar, As(V). The proportions of As chemical species in the total extract are shown in Table 1. Although a fraction of As was absorbed by the cells, As concentrations in solution were very high and the production of biomass after 30 days (0.4 g L⁻¹) was insufficient to significantly reduce the concentration of total As in the supernatant (t test for paired means, $p>0.05$) (Table 2). However, the total As in biomass exhibited concentration of $\sim 60.0 \mu\text{g g}^{-1}$.

As coordination

Overlapping X-ray absorption near-edge structure (XANES) spectrum of aqueous and crystalline standards of As(III) and As(V) suggest similar local As environments in oxyanions with the same As valence regardless of the state of the compound

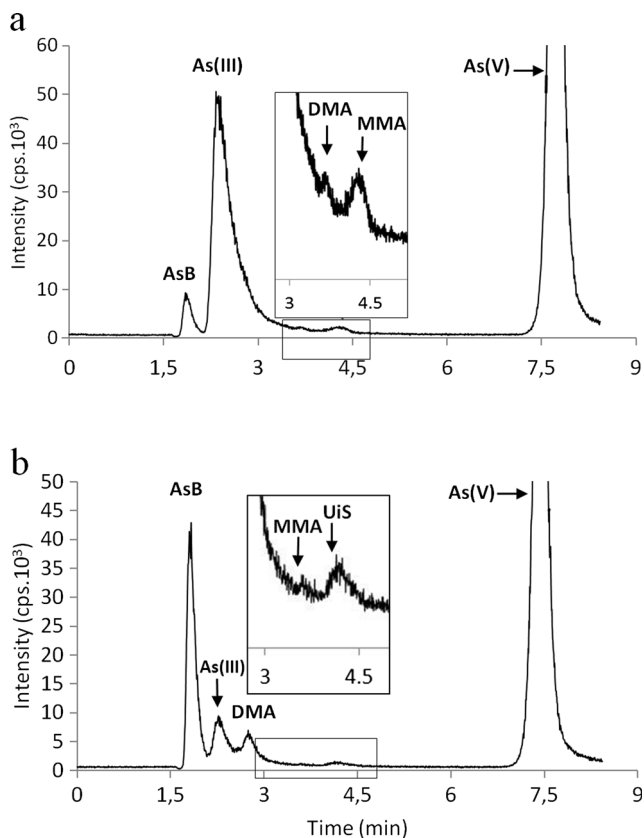


Fig. 3 Speciation analysis of *Synechococcus* sp. biomass exposed to a As(V) and b As(III). An expansion of the As species observed at a retention time of 3 to 4.5 min is shown

(Fig. 4a). A Fourier transform (FT) of the extended X-ray absorption fine structure (EXAFS) region of the spectrum revealed that an As K-edge in the supernatant from the cultures exposed to As(V) overlapped with the one obtained from the As(V) aqueous pattern, indicating that As(V) [H₂AsO₄ or HAsO₄⁻²] was the predominant As species in the supernatant (Fig. 4b). Other As chemical species detected by HPLC-ICP-MS in the supernatant were present at concentrations that were insufficient to produce variations in the EXAFS oscillations.

The As K-edge XANES spectra obtained from aqueous standards covalently bonded to carbon, showed that oxygen and sulfur are candidate atoms for the intracellular As

coordination, together with the spectrum for the biomass under exposure to As(V) (Fig. 5a). By inspection, it can be seen that the energy edge of the As(V)-exposed biomass sample lies somehow in between the edges for As(III) and As(V) standards, suggesting a mixture of both species. To evaluate this hypothesis, a linear combination of experimental As(III) and As(V) standards was fitted to the XANES spectrum of lyophilized cells exposed to As(V) and the fit revealed that 34 % of the intracellular As was reduced to As(III) and 66 % remained As(V) (Fig. 5b).

Discussion

The EC₅₀ tests demonstrated that As(III) is approximately 400-fold more toxic than As(V) to *Synechococcus* sp., confirming that As toxicity in aquatic microorganisms is strongly related to its oxidation state. As demonstrated in previous studies, As(III) is at least 5-fold more toxic than As(V) for cyanobacteria. *Microcystis aeruginosa* exhibited a decreased growth rate after exposure to As(III) at concentrations higher than 0.75 mg L⁻¹. However, no inhibition of growth was observed at concentrations of As(V) up to two orders of magnitude higher (Gong et al. 2011). A similar result was reported for *Anabaena doliolum* which exhibited EC₅₀ of 4345 mg L⁻¹ for As(V) and 824 mg L⁻¹ for As(III) (Srivastava et al. 2009). Thus, in addition to the redox state, As toxicity is strongly influenced by factors inherent to the cyanobacteria species.

Chemical and structural differences between As(III) and As(V) can produce specific intracellular effects. As(III) can denature proteins by binding to sulfhydryl groups (Shen et al. 2013), whereas As(V) uncouples oxidative phosphorylation (Gresser 1981) and replaces the phosphate groups of lipid membranes (Tuan et al. 2008). The absorption of As(V) is related to phosphate biochemistry as it competes for phosphate trans-membrane transporters, and for As(III), the proposed membrane route of entry is the aquaglyceroporins, a family of proteins present in cyanobacteria that are responsible for the transport of neutral molecules such as glycerol (Liu

Table 1 Proportions of As chemical species as percentages of the total extract

As added	Extracted fraction	AsB	As(III)	DMA	MMA	UiS species	As(V)
As(III)	Intracellular	12.11±2.84	6.28±1.79	3.49±0.62	1.01±0.13	0.86±0.15	76.21±4.83
	Supernatant	0.14±0.01	–	0.14±0.01	0.42±0.08	0.38±0.24	98.82±0.14
	Control	–	–	–	–	–	100
As(V)	Intracellular	2.62±0.35	34.97±1.99	–	2.20±0.05	–	60±2.28
	Supernatant	–	–	0.13±0.06	–	–	99.86±0.06
	Control	–	–	–	–	–	100

Values are given in average±SE

Table 2 Total As concentrations in the supernatant, control (initial and final), and in biomass

Tests	[As] nominal (mg L ⁻¹)	[As] initial (mg L ⁻¹)	[As] final (mg L ⁻¹)	[As] biomass (μg g ⁻¹)
As(III) + <i>Synechococcus</i> sp.	6	5.83±0.05	5.78±0.01	61.61±8.43
As(III) control	6	6.09±0.01	5.87±0.02	–
As(V) + <i>Synechococcus</i> sp.	400	416.30±4.75	421.46±19.91	60.11±1.37
As(V) control	400	404.86±1.42	402.72±4.19	–

Values are given as the average±SE

et al. 2002). These biochemical differences according to As oxidation state may influence the amount of As absorbed and also the differences between As(III) and As(V) toxicity. Even though the cells were exposed to concentrations of As(III) approximately 67-fold lower than those of As(V), similar intracellular concentrations of As were observed after 30 days of exposure (Table 2). In cells of *Microcystis aeruginosa*, Wang et al. (2013) observed higher As(III) uptake compared to As(V) when the cells were exposed to same concentrations.

The growth rate of *Synechococcus* sp. after exposure to As(V) and As(III) decreased in the early stages and was taken up after 24 and 72 h, respectively (Fig. 1). This behavior suggests an initial cytostatic effect that may be counterbalanced by cell resistance mechanisms such as changes in gene expression and the activity of enzymes like arsenate reductase (Pandey et al. 2012). Similar to the response of *Synechococcus* sp. to As(III) exposure, *M. aeruginosa* resumed normal growth after 7 days of exposure to 3.8 mg L⁻¹ As(III) (Wang et al. 2013). *Synechococcus* sp. exhibited behavior similar to that of *Anabaena* sp. after exposure to an EC₅₀ of 3000 mg L⁻¹ As(V), which resumed growth after 24 h (Pandey et al. 2012).

The detoxification pathway in which As(V) undergoes steps of reduction intercalated with the oxidative methylation to produce trimethylarsine (TMA) as the end-product was proposed by Challenger (1945) (As⁵⁺→As³⁺→MMA⁵⁺→MMA³⁺→DMA⁵⁺→DMA³⁺→TMAO⁵⁺→TMA³⁺). Since then, it has been proved experimentally that Challenger steps of As biotransformation are universal (Hughes et al. 2011). Recently, the production of TMAO was also demonstrated in cyanobacteria (Yin et al. 2011). Here, we found that after As(V) exposure, the most abundant As species were As(V) > As(III) > organic As, whereas after As(III) exposure, the prevalence of As-species were As(V) > organic As > As(III) (Table 1).

Figure 3 shows quantitative and qualitative information regarding intracellular As species. After As(V) exposure (Fig. 3a), the higher production of As(III) is possibly a result of arsenate reductase activity on the first step of Challenger detoxification pathway. Alternatively to the methylation step, As(III) could be extruded of the cells by the As specific efflux pump (Paez-Espino et al. 2009). On the other hand, one possible explanation for the higher production of several As

organic species, after As(III) exposure (Fig. 3b), is that As(III) can be readily methylated, decreasing its intracellular levels. Furthermore, As(III) complexation with Cys-rich peptides also contribute to decrease As(III) levels.

It is generally accepted that the biological oxidation of As(III) could be a detoxification mechanism, as As(V) is less noxious (Paez-Espino et al. 2009; Chang et al. 2010). As(III) oxidation by cyanobacteria has been demonstrated (Wang et al. 2013; Yin et al. 2012) and it is consistent with the

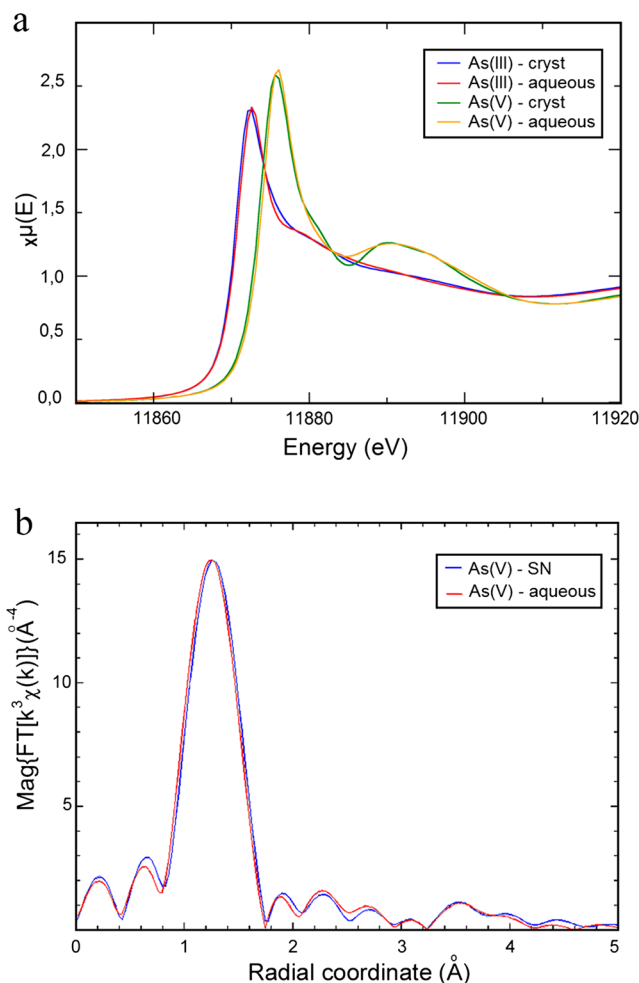


Fig. 4 **a** XANES spectra of the As K-edge obtained from aqueous and crystalline (cryst.) patterns of As(III) and As(V). **b** Fourier transform of the EXAFS oscillations for the As(V) aqueous pattern and the supernatant [As(V)-SN] of cultures exposed to As(V)

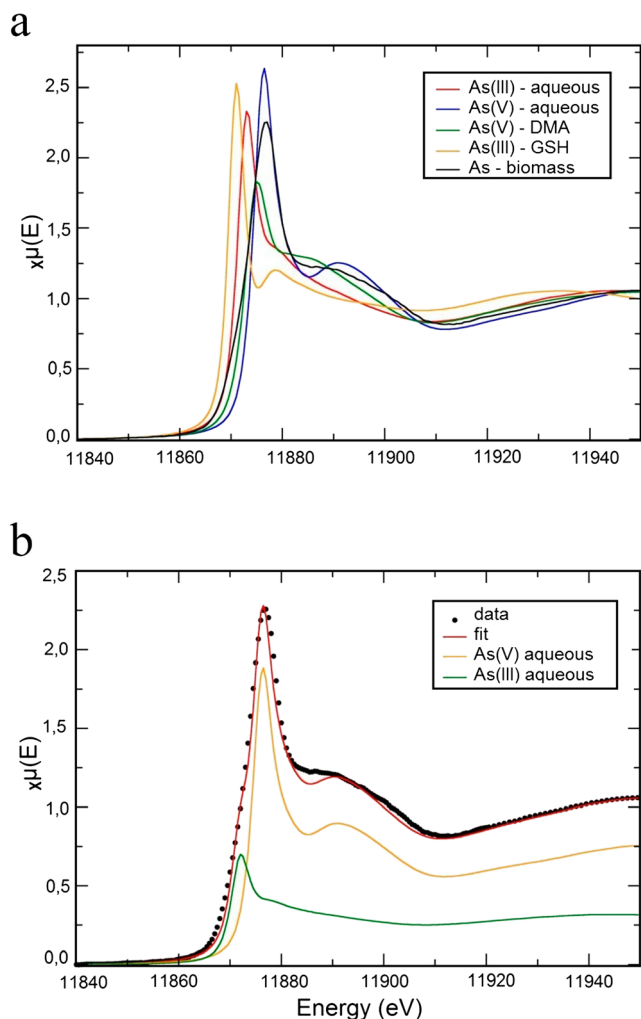


Fig. 5 a XANES spectra of the As K-edge obtained from intracellular As (biomass) and the As(III), As(V), DMA, and As(III)+GSH patterns for As(V)-O, As(III)-O, As-C, and As-S bonds, respectively. b The linear combination of As(V) and As(III) spectra indicated contributions of 66 and 34 %, respectively, to the As K-edge spectrum of intracellular As

As(V) predominance observed within cells. The As(V) intracellular dominance, followed by As(III) and biomethylated species in minor quantities (less than 5 %) were also demonstrated in studies with other cyanobacteria species (Wang et al.

2013; Yin et al. 2011), regardless of whether the initial oxidation state was As(III) or As(V) (Table 3). Furthermore, the process of biomethylation is also favored by high As concentrations and longtime exposure (Wang et al. 2013; Yin et al. 2011, 2012).

Changes in the redox state and methylation are the major mechanisms of As detoxification found in cyanobacteria (Table 3). DMA and other As species were also detected in the supernatant, supporting the role of methylation in the extrusion of As from the cells (Table 1). According to Rahman and Hassler (2014), biomethylation is considered an effective detoxification mechanism if the microorganism predominantly produces less toxic pentavalent species: MMA(V), DMA(V), and trimethylarsine [TMAO(V)] compared with the toxic trivalent species [MMA(III) and DMA(III)]. After As(III) exposure, increasing in organic As concentrations and the presence of unidentified organic species (UiS) were observed. Among the organic As species, one exhibited the same retention time of arsenobetaine (AsB) (C₅H₁₁AsO₂), a zwitterion with a positive charge on the As atom and a negative charge on the carboxyl group (Fig. 3b). AsB is the main As excretion product in zooplankton and animals and is also produced by macroalgae (Caumette et al. 2012). However, this As chemical species has not previously been detected in cyanobacteria. More specific techniques are required for the identification of AsB once the anion exchange column used is suitable for separating anionic species, including As(V), MMA, DMA, and As(III) (Leermakers et al. 2006).

As(V) was the major species in both the supernatant and the algae free-control of As(III) after 30 days (Table 1). The stability of As(III) depends on physical and chemical characteristics of the media like pH, oxygen concentration, and presence of oxidative compounds (Bissen and Frimmel 2003; Samanta and Clifford 2005). In previous studies, the oxidation of As(III) in the supernatant in a shorter period of time (14 or 15 days) was attributed to the cyanobacteria activity as part of detoxification mechanism (Wang et al. 2013; Yin et al. 2012). Because As(III) oxidation may occur in both intracellular and extracellular environments, it is necessary to perform speciation analysis in the supernatant

Table 3 Comparison of As species (in percentage) in cells of some cyanobacteria species after exposure to As oxyanions

Cyanobacteria species	As exposed: species/concentration	Time (days)	As(V)	As(III)	DMA+MMA	AsB	Reference
<i>Synechococcus</i> sp.	As(III) 6 mg L ⁻¹	30	76	6.2	4.5	12.1	This work
	As(V) 400 mg L ⁻¹	30	60	34.9	2.2	2.5	
<i>Synechocystis</i> sp. <i>Microcystis</i> sp.	As(III) 7.5 mg L ⁻¹	14	80 ^a	16 ^a	3 ^a	–	Yin et al. (2011)
			96 ^a	2 ^a	1 ^a		
<i>Nostoc</i> sp.			89 ^a	7 ^a	3 ^a		
<i>M. aeruginosa</i>	As(III) 3.75 mg L ^{-1b}	15	81 ^a	5 ^a	<1 ^a	–	Wang et al. (2013)
	As(V) 7.5 mg L ^{-1b}	15	77 ^a	11 ^a	<1 ^a		

^a Concentrations estimated according to the values presented in results

^b Maximum concentration exposed

in shorter periods of time (daily) to assign arsenic oxidation specifically to *Synechococcus* sp.

The sum of the different As species fractions in the supernatants and controls resulted in an average of 104 ± 13 % compared to total As determined by ICP-OES (Table 2). A fraction of 5 % of the total arsenic present in the biomass was extracted. This low yield was not an issue for speciation, given the low detection limit (40 fg/g) of the equipment (HPLC-ICP-MS). Similarly, a 10 % extraction yield was observed for *Euglena gracilis* and attributed to the solubility of As chemical species in the extraction solution or the association of a fraction of the As with the membranes (Halter et al. 2012).

Synchrotron radiation-based techniques have been used in studies of As speciation in biological samples (Pérez et al. 2014). The XANES and EXAFS techniques can be used to provide information on the oxidation state and to determine the local coordination of a target chemical, respectively. These techniques preserve and determine the proportions of redox state and coordination of the target element. The adjustment for the As K-edge spectrum of the *Synechococcus* sp. biomass (Fig. 5b) after exposure to As(V) was consistent with the proportions of the chemical species determined by HPLC-ICP-MS. However, the determination of the coordination by XAFS has limitations, including a high limit of detection and energy intensity of the light beam from the synchrotron radiation source. Even with a large number of scans at low temperatures the low signal-to-noise ratio obtained precluded the determination of the coordination of As.

In this study, the differential toxicity of As(III) and As(V) demonstrated by *Synechococcus* sp. indicates that evaluating the redox state of As is essential to assess the risk of As toxicity in aquatic organisms. Furthermore, Rahman and Hassler (2014) have discussed that the redox state of methylated As compounds resulting from the detoxification process is crucial to determine their final toxicity, being less toxic when these final products are pentavalent forms. In conclusion, *Synechococcus* sp. can absorb and transform inorganic As species into methylated and other organic As species, confirming the hypothesis that *Synechococcus* sp. has the ability to biotransform As oxyanions. When exposed to As(III), lower concentrations of As(III) intracellular and higher proportions of methylated species were observed compared to As(V) exposure. These biotransformation processes are likely associated to As detoxification. The use of XANES and HPLC-ICP-MS allowed the unambiguous identification of the As redox states and the properties of *Synechococcus* sp. to biotransform As oxyanions.

These data provide a foundation for future studies of this biological model under environmentally relevant conditions to evaluate the effects of As on the metabolism of *Synechococcus* sp., to understand the role of cyanobacteria in the biogeochemical cycle of As and finally, use this

promising potential feature for remediation of As environmental contamination.

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Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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