

Potential of *Synechococcus* sp. (Cyanobacteria) for arsenic biotransformation: evidences from culture experiments

M.W. Franco^{1*}, F.A. Guedes¹, I.F. Vasconcelos², B.L. Batista³, D.G.F. Pujoni¹, S.M.S. Magalhães⁴, F. Barbosa Jr⁵, F.A.R. Barbosa¹

¹Laboratory of Limnology, Ecotoxicology and Aquatic Ecology - LIMNEA, Institute of Biological Sciences, Universidade Federal de Minas Gerais-UFMG, Belo Horizonte, MG, Brasil.

²Department of Metallurgical and Materials Engineering, Universidade Federal do Ceará, Campus do Pici Bloco 714, Fortaleza 60455-760, CE, Brasil.

³ Center of Natural Sciences and Humanities, Universidade Federal do ABC, Santo André 09210-580, SP, Brasil.

⁴Department of Social Pharmacy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.

⁵Laboratory of Toxicology and Essentiality of Metals, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto 14040-903, SP, Brasil.

*Corresponding author: barbosa@icb.ufmg.br – Av. Antônio Carlos, 6627 ZIP Code 30.270-901, Belo Horizonte, MG.

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The biogeochemistry of As is complex and involves transformation into various chemical species with different levels of toxicity. In lakes, phytoplankton blooms activity has a strong influence on As availability and speciation (Hellweger and Lall 2004). 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. 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). Molecules containing cysteine residues such as the tripeptide glutathione (γ -L-glutamyl-L-cysteinylglycine, GSH) are critical in this process because of the high As(III) affinity for sulfhydryl groups. Arsenic metabolism by cyanobacteria has mainly been studied in water-bloom-forming species from eutrophic lakes. 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 compounds formed by *Synechococcus* sp., a non-toxic and non-bloom-forming cyanobacterial strain obtained from a stream in a mining area contaminated with As (average 0.06 mg L⁻¹). The aim of this study was to evaluate the biotransformation of As by *Synechococcus* sp.

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). As speciation was performed by high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and X-ray absorption fine structure spectroscopy (XAFS) at Laboratório Nacional de Luz Síncrotron. The intracellular levels (60.0 mg g⁻¹) of As were the

same after 30 days of exposure to As(V) and As(III). After As(V) exposure the most abundant As species were As(V) > As(III) > Organic As, whereas after As(III) exposure were As(V) > Organic As > As(III) (Table 1). Among the organic As species observed, one exhibited the same retention time of arsenobetaine (AsB) ($C_5H_{11}AsO_2$), a zwitterion with a positive charge on the As atom and a negative charge on the carboxyl group (Figure 1).

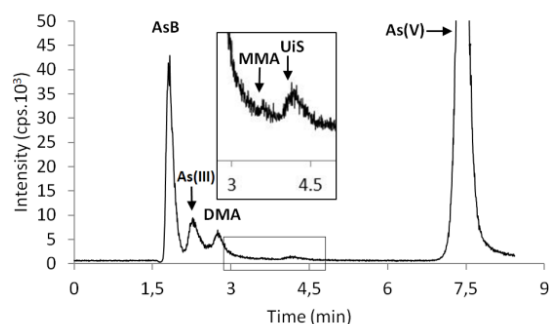


Figure 1. Speciation analysis of *Synechococcus* sp. biomass exposed to As(III). An expansion of the As species observed at a retention time of 3 to 4.5 min is shown.

Arsenobetaine is considered to be non-toxic containing a single arsenic atom in the +5 oxidation state bound to three methyl groups and one acetic acid group. This As compound is the main As excretion product in zooplankton and fish, also found in 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. 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), in *Synechococcus* sp. production of organic species occurred in higher proportions, notably after As(III) exposure (Table 1), probably because As(III) can be readily methylated and presents affinity to protein sulfhydryl groups.

Arsenic K-edge XANES spectra were obtained from aqueous standards of different compounds of

As (As(V), As(III), As(III)+GHS and DMA) (Figure 2A) and compared to the spectra obtained from biomass samples exposed to As(V). It was found that oxygen and sulfur are the possible components of the intracellular As coordination. A linear combination of 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) (Figure 2b). These results were consistent with the proportions of the chemical species determined by HPLC-ICP-MS.

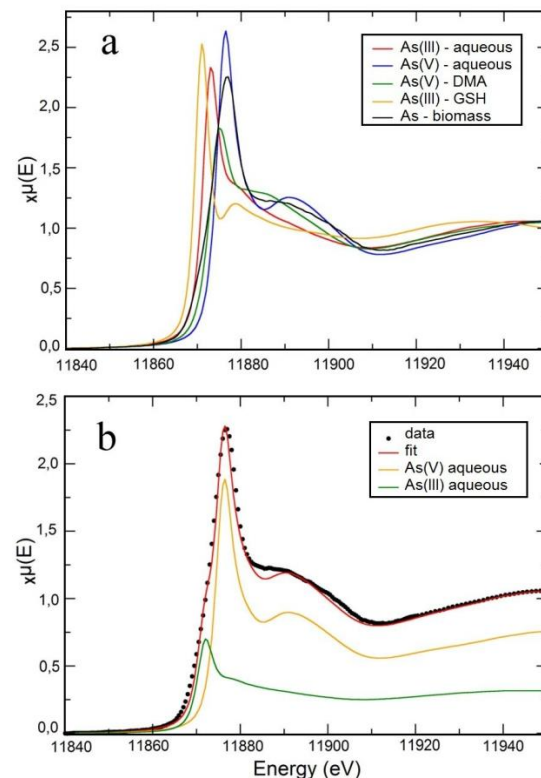


Figure 2. (a) XANES spectra of the As K-edge obtained from intracellular As (biomass) and the As(III), As(V), DMA and As(III)+GSH patterns for As(V)-O, As(III)-O, As-C and As-S bonds, respectively. (b) The linear combination of As(V) and As(III) spectra indicated contributions of 66% and 34% respectively to the As K-edge spectrum of intracellular As.

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
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

Values are given in average ± SE. *UiS unidentified As species

Table 2. Comparison of As species (i%) in cells of distinct 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.			80*	16*	3*	-	Yin et al. 2011
<i>Microcystis</i> sp.	As(III) 7.5 mg L ⁻¹	14	96*	2*	1*		
<i>Nostoc</i> sp.			89*	7*	3*		
<i>M. aeruginosa</i>	As(III) 3.75 mg L ^{-1**}	15	81*	5*	< 1*	-	Wang et al. 2013
	As(V) 7.5 mg L ^{-1**}	15	77*	11*	< 1*		

*Concentrations estimated according to the values presented in results. **Maximum concentration exposed.

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. Changes in the redox state and methylation are the major mechanisms of As detoxification found in cyanobacteria (Table 2). The use of XANES and HPLC-ICP-MS allowed the unambiguous identification of the As redox states and biotransformation properties of *Synechococcus* sp. 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 to use this promising potential of As biotransformation for remediation of As in environmental contamination.

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