JOURNAL OF ENVIRONMENTAL SCIENCES XX (2017) XXX–XXX

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JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

Arsenate biotransformation by *Microcystis aeruginosa* under different nitrogen and phosphorus levels

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ARTICLE INFO

Article history:
Received 17 February 2017
Revised 27 May 2017
Accepted 27 May 2017
Available online xxxx

Keywords:
Arsenate
Biotransformation
Nitrogen
Phosphorus
M. aeruginosa

ABSTRACT

The arsenate (As(V)) biotransformation by *Microcystis aeruginosa* in a medium with different concentrations of nitrogen (N) and phosphorus (P) has been studied under laboratory conditions. When 15 μg/L As(V) was added, N and P in the medium showed effective regulation on arsenic (As) metabolism in *M. aeruginosa*, resulting in significant differences in the algal growth among different N and P treatments. Under 0.2 mg/L P treatment, increases in N concentration (4–20 mg/L) significantly stimulated the cell growth and therefore indirectly enhanced the production of dimethylarsinic acid (DMA), the main As metabolite, accounting for 71%–79% of the total As in the medium. Meanwhile, 10–20 mg/L N treatments accelerated the ability of As metabolization by *M. aeruginosa*, leading to higher contents of DMA per cell. However, As(V) uptake by *M. aeruginosa* was significantly impeded by 0.5–1.0 mg/L P treatment, resulting in smaller rates of As transformation in *M. aeruginosa* as well as lower contents of As metabolites in the medium. Our data demonstrated that As(V) transformation by *M. aeruginosa* was significantly accelerated by increasing N levels, while it was inhibited by increasing P levels. Overall, both P and N play key roles in As(V) biotransformation processes.

Introduction

Arsenic (As) is a strongly carcinogenic metalloid which is widely distributed in soils, rocks, and natural waters, and has attracted widespread concern due to its severe threat to ecosystem health (Singh et al., 2007). Recently, because of increasing anthropogenic pollution, As contamination in freshwater has gradually become a serious environmental issue in the world (Rahman et al., 2014). The toxicity of As to organisms in freshwater is related to its species and concentration. In general, inorganic As species are more prevalent and more toxic than organoarsenicals (Meharg and Hartley-Whitaker, 2002; Smedley and Kinniburgh, 2002). Arsenate (As(V)) tends to be the dominant species in oxic conditions, while arsenite (As(III)) is predominant in reducing environments (Hasegawa et al., 2010). The dominant organic forms (monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA)) are detected at lower levels in aquatic ecosystems (Akter et al., 2005). It has been shown that conversion of As species primarily depends on the microorganisms in natural ecosystems (Cullen and Reimer, 1989; Levy et al., 2005), thus it is crucial to understand As biotransformation by microorganisms in order to predict its ecological risk in As-contaminated freshwater.

As the important primary producers and prevalent inhabitants of aquatic systems, algae play a vital role in As biogeochemical cycling because they show tolerance toward As and are capable of metabolizing it along several pathways (Levy et al., 2005; Pawlik-Skowronska et al., 2004; Ye et al., 2012; Yin et al., 2012). Interestingly, the ability for As metabolization in algae is related to various environmental factors, including nutrient status, temperature, light intensity, pH, *etc.* (Wang et al., 2015).

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http://dx.doi.org/10.1016/j.jes.2017.05.041
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Among these factors, nitrogen (N) and phosphorus (P) are essential elements for various metabolic processes in algal cells, thus the nonnegligible effects of N and P on As biotransformation by algae should be taken into consideration. Previous studies have already manifested that P can effectively affect As toxicity, uptake and efflux, redox processes, and methylation in algae (Guo et al., 2011; N.X. Wang et al., 2013; Z.H. Wang et al., 2014; Yan et al., 2014; Zhang et al., 2014). In addition to regulating algal growth, N is also considered an indispensable element to stimulate the synthesis of antioxidants, which can defend against environmental stresses caused by heavy metals (Downing et al., 2005; Liu et al., 2015a; Shao et al., 2009). Therefore, altered N concentration has the potential to affect the tolerance to pollutants in algae. It has been reported that As accumulation in *Nostoc* sp. (Maeda et al., 1993) and *Chlamydomonas reinhardtii* (N.X. Wang et al., 2014) may change under different NO_3^- concentrations in culture. However, to the best of our knowledge, little has been studied regarding the potential influence of N on As biotransformation in algae. Even less is known about the combined influence of N and P on As biotransformation.

Microcystis aeruginosa, as typical freshwater cyanobacteria, are widely distributed in the environment, and are dominant in blue-algal blooms, especially in freshwater with higher trophic status (Oberholster et al., 2004). In view of the coexistence of cyanobacterial blooms and As contamination in many freshwater lakes around the world, it is important to reveal the combined effects of N and P on As biotransformation. In this study, we chose the strain *M. aeruginosa* (FACHB 905) to study the influences of N and P on As biotransformation processes. The contents of different As species both in algae and in culture, as well as the As metabolism pathway in *M. aeruginosa*, were investigated under different concentrations of N and P. Our results can contribute to a better understanding of N and P-regulated interactions between As contaminants and cyanobacteria, and facilitate further understanding of As biogeochemistry in aquatic environments.

1. Materials and methods

1.1. Cultivation and growth condition

The axenic strain of *M. aeruginosa* (FACHB 905), purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, was cultivated in sterile BG11 medium in Erlenmeyer flasks and used throughout the experiment. Sterile precautions were taken with all procedures requiring handling during the experiment to ensure that algal cultures were free from microbial contamination, including ethanol sterilization of the clean bench, open flame sterilization on inoculation, and autoclaved glassware and medium. All the apparatus and the medium used for algal culture and experiments were autoclaved at 121°C for 30 min, and were handled under sterile conditions prior to use. The axenic status of a culture can be confirmed by streaking on an agar plate followed by incubating for 3 days. A bacteria-free algal culture was obtained since no bacteria were observed on the agar algal plate. The axenic *M. aeruginosa* cultures were maintained in a controlled-environment growth chamber under the following conditions: 16:8 light–dark cycle with light intensity of 115 μmol photons/(m^2·sec) at 25°C.

1.2. Experimental design

Cells of *M. aeruginosa* in the exponential growth phase were collected by centrifugation at 9000 r/min at 8°C for 15 min, rinsed with sterile Milli-Q water to remove N and P on the algal surface, and then cultured in sterile BG11 without N and P for 48 hr to consume the remaining N and P *in vivo*. In order to investigate As biotransformation on a practical level in aquatic systems, the selected As(V) concentration (15 μg/L) in this study was similar to those measured in natural aquatic systems under low pollution levels (Smedley and Kinniburgh, 2002; Yan et al., 2016). The effects of N and P were investigated by comparing As(V) metabolization in *M. aeruginosa* cultures, enriched with 15 μg/L As(V) ($Na_3AsO_4{\cdot}12H_2O$, Fluka, p.a.) and specific concentrations of N ($NaNO_3$) and P (K_2HPO_4) as shown in Table 1. Algal cultures without As(V) added were used as control. In addition, an algae-free medium with 15 μg/L As(V) added was prepared to investigate the As abiotic transformation in an axenic environment. The nutrient treatments were selected according to the concentration ranges of N and P in freshwater with different trophic levels and the relationship of N:P ratio reported in previous research (Dos Anjos et al., 2012; Downing and Mccauley, 1992; Paerl et al., 2011; Xie et al., 2003; Xu et al., 2010).

Each experiment was replicated three times and lasted for 8 days. The initial cell density of algal cultures was controlled at 10^6 cells/mL. The flasks were shaken well three times every day and before each sampling. In order to determine As absorption and transformation in *M. aeruginosa*, 10-mL aliquots of algal cultures from each replicate were harvested after exposure to As(V) for 2, 4, 6, and 8 days. Meanwhile, 1.5-mL aliquots of medium were collected each day for the investigation of As excretion into the medium. In addition, the daily cell density of *M. aeruginosa* was determined for the observation of algal cell growth.

1.3. Total as determination

For the determination of intracellular total As (TAs) concentration, the collected algal samples were rinsed with Milli-Q water and ice-cold phosphate buffer (1 mmol/L K_2HPO_4, 5 mmol/L MES, and 0.5 mmol/L $Ca(NO_3)_2$) for 15 min to remove apoplastic As. Then, the freeze-dried algae were kept in 2-mL tubes with 1 mL of concentrated HNO_3 (65%, guaranteed reagent) overnight in preparation for TAs digestion. Briefly, the sample digestion was completed using a microwave accelerated reaction system (MARS-Xpress, CEM Microwave Technology Ltd., USA), with the digestion program as follows: 55°C for 10 min, 75°C for 10 min,

Table 1 – The initial concentrations of nitrogen (N) and phosphorus (P) for five treatments in the test medium.

Experiment	Initial concentration (mg/L)				
	4:0.2	10:0.2	20:0.2	10:0.5	10:1.0
N ($NaNO_3$)	4	10	20	10	10
P (K_2HPO_4)	0.2	0.2	0.2	0.5	1.0
N:P ratio	20	50	100	20	10

Please cite this article as: Che, F., et al., Arsenate biotransformation by *Microcystis aeruginosa* under different nitrogen and phosphorus levels, J. Environ. Sci. (2017), http://dx.doi.org/10.1016/j.jes.2017.05.041