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Impacts of environmental factors on arsenate biotransformation and release in *Microcystis aeruginosa* using the Taguchi experimental design approach

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ABSTRACT

Very limited information is available on how and to what extent environmental factors influence arsenic (*As*) biotransformation and release in freshwater algae. These factors include concentrations of arsenate (*As*(V)), dissolved inorganic nitrogen (N), phosphate (P), and ambient *pH*. This study conducted a series of experiments using Taguchi methods to determine optimum conditions for *As* biotransformation. We assessed principal effective factors of *As*(V), N, P, and *pH* and determined that *As* biotransformation and release actuate at 10.0 μM *As*(V) in dead alga cells, the *As* efflux ratio and organic *As* efflux content actuate at 1.0 mg/L P, algal growth and intracellular arsenite (*As*(III)) content actuate at 10.0 mg/L N, and the total sum of *As*(III) efflux from dead alga cells actuates at a *pH* level of 10. Moreover, *N* is the critical component for *As*(V) biotransformation in *M. aeruginosa*, specifically for *As*(III) transformation, because *N* can accelerate algal growth, subsequently improving *As*(III) accumulation and its efflux, which results in an *As*(V) to *As*(III) reduction. Furthermore, low *P* concentrations in combination with high *N* concentrations promote *As* accumulation. Following *As*(V), *P* was the primary impacting factor for *As* accumulation. In addition, small amounts of *As* accumulation under low concentrations of *As* and high *P* were securely stored in living algal cells and were easily released after cell death. Results from this study will help to assess practical applications and the overall control of key environmental factors, particularly those associated with algal bioremediation in *As* polluted water.

1. Introduction

Arsenic (*As*), a ubiquitous, toxic, and carcinogenic metalloid, is present in the environment in considerable quantities both naturally and as a result of certain human activities. It resides in the environment in four primary oxidation states (−3, 0, +3, and +5) under different physicochemical properties. Furthermore, *As* pollution in freshwater systems is one of the most common global environmental problems with approximate concentrations ranging from 0.5 to 5000 μg/L (Smedley and Kinniburgh, 2002; Pfeiffer et al., 2015; Luo et al., 2014). Arsenite (*As*(III)) and arsenate (*As*(V)) are the common inorganic forms of *As* found in natural freshwater systems, the latter being the dominant form under oxic conditions (Hasegawa et al., 2010). This has prompted numerous studies investigating the distribution and behavior of inorganic *As* in water bodies. These studies have primarily focused on mechanisms related to *As* metabolism and resistance in microorganisms and bioremediation in *As* polluted water bodies (Smedley and Kinniburgh, 2002; Wang et al., 2013a; Jasrotia et al., 2014).

Being at the bottom of the aquatic food chain, alga are widely distributed in aquatic ecosystems and play an important role in *As* bioaccumulation and biogeochemical cycling (Duncan et al., 2015; Zhang et al., 2014). At the same time, given the extremely high uptake capacity of alga as well as the fact that alga are more cost-effective and environmentally-friendly than the conventional physicochemical methods used, employing alga in remediation efforts of *As* polluted water has garnered considerable attention (Bahar et al., 2013; Sulaymon et al., 2013; Mahdavi et al., 2012; Wang et al., 2015). Moreover, a pervasive phenomenon

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commonly seen today is that eutrophication coexists with *As* contamination in freshwater ecosystems (Le et al., 2010; Sun, 2004). Occasionally, harmful blooms of *Microcystis aeruginosa* (*M. aeruginosa*) outbreaks occur, which are toxic to both plants and animals (Jasrotia et al., 2014). This species is generally tolerant to *As*(V) and exhibits a stronger *As* bioaccumulation capacity compared to other freshwater algae (Hasegawa et al., 2010). It has a favorable capacity in manipulating *pH* homeostasis, and to a certain extent it can affect *pH* levels in water to favor its own growth (Pang et al., 2013). Such characteristics make it an ideal candidate to be considered as a potential biosorbent (Rzymski et al., 2014; Sun et al., 2014). Since microalgae research is important for both the phytoremediation of wastewater and pollution prevention of algal products, an in-depth understanding of *As* metabolic functions in microalgae is urgently needed.

Many abiotic factors affect the metabolic functions of alga contaminated by *As*, such as *As* levels (Gong et al., 2009; Hasegawa et al., 2001), hydrogen ion levels (*pH*) (Hasegawa et al., 2001; Zhang et al., 2013), and key nutrient concentrations of nitrogen (*N*) and phosphorus (*P*) in culture media (Hasegawa et al., 2001; Wurl et al., 2013; Lei et al., 2012; Wang et al., 2014a). Moreover, the similar chemical structures between PO_4^{3-} and AsO_4^{3-} do not only influence algal growth but also promote competitive behavior in the way in which they uptake, accumulate, and release ingested arsenical compounds (Wang et al., 2013a, 2014b). Biotransformation of *As*(V), which occurs via the intracellular methylated *As* content of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in alga, can also be significantly impacted by changes in *P* content in media (Wang et al., 2013a; Duncan et al., 2015; Karadjova et al., 2008; Guo et al., 2011; Foster et al., 2008).

It has also been suggested that *N* content in growth media can affect *As* accumulation in algal cells. Wang et al. (2014a) reported that *As*(III) transporters in *Chlamydomonas reinhardtii* were unaffected by nitrate in the medium, but they observed induced synthesis under *N*-limited cells. Additionally, Maeda et al. (1993) reported that *As*(V) accumulation by *Nostoc* sp. decreased with an increase in ambient *N* concentrations. Additionally, *pH* levels in aquatic environments can influence *As* biosorption, which plays an important role in *As* detoxification for various algal species (Zhang et al., 2013; Ma et al., 2015). An interconversion between *As*(III) and *As*(V) may also occur under different *pH* conditions (Smedley and Kinniburgh, 2002; Bears et al., 2006). Moreover, it was found that *As* accumulation in *Vallisneria natans (Lour.) Hara* increased under increasing *pH* levels (Chen et al., 2014a). Additionally, *pH* can affect *As* methylation (Sadiq, 1997). Maeda et al. (1992) observed that *pH* affected both total *As* (T*As*) concentrations and relative concentrations of methylated *As* excretions from *Chlorella vulgaris* cells that accumulated in the presence of *As*.

The factors mentioned above have been separately studied under controlled conditions in laboratories using much higher concentrations than those that normally exist in uncontaminated aqueous environments (Duncan et al., 2013). To date, there are only a limited number of available studies related to effects of metabolic *As* functions in alga, particularly those associated with *As*(V). Given that they are the essential environmental factors that influence both algal growth and *As* metabolism as well as integrated and systematic effects of *As*(V) levels, *N* and *P* concentrations, *pH* levels on algal growth, and *As* metabolism itself, these metabolic functions associated with *As* in alga remain unclear and require further investigation (Wang et al., 2015). To further understand environmental factors that impact *As*(V) uptake, we investigated *As* biotransformation and release in *M. aeruginosa*, aspects of its growth, intracellular *As* accumulation in algae cells, and release after algae death. Furthermore, to decrease the number of experiments while evaluating impacts of each parameter independently, we applied Taguchi methods under their relevant statistical assumptions to determine optimum environmental conditions for *As* biotransformation and release in *M. aeruginosa*. The method we used in this study is favorable for experimental replications that are concerned only with key effects of design parameters (Zolfaghari et al., 2011; Rao et al., 2008).

2. Materials and methods

2.1. Experimental design

To study the effects of the four controllable parameters (*As*(V), *pH*, *N*, and *P*) on *M. aeruginosa* growth as well as the metabolic functions of *As*, we used Taguchi methods to identify optimal conditions as well as to determine key parameters that influence algal growth, *As*(V) biotransformation, and *As* efflux from dead algal cells. In this study, these four factors were assumed to be independent under Taguchi methods. The most important feature of Taguchi methods is its application of an orthogonal array to determine parameters of controllable factors with the aim to minimize impacts of uncontrollable factors (noise) (Chen et al., 2014b). Therefore, it allows for analyses which prioritize comparative impacts of these factors on algal growth, *As*(V) biotransformation, and *As* efflux from dead algal cells.

In this study, three different levels of each environmental factor represent low, intermediate, and high pollution levels of nutrients, *pH*, and *As* under real-world aquatic conditions (Yan et al., 2016). As shown in Table 1, we selected levels of *N* and *P* according to the maximum values of surface water environmental quality standards in China to represent mesotrophic, eutrophic, and hypereutrophic aquatic systems (Rahman and Hasegawa, 2012). In addition, the *pH* levels we selected represent actual *pH* freshwater ranges, and *As*(V) concentrations were similar to those measured in natural aquatic systems under low, intermediate, and high pollution levels (Yan et al., 2016; Caumette et al., 2011). Based on the Taguchi design concept, we selected the L9 (3^4) orthogonal array, and experimental conditions were obtained (Table 2) by combining the information provided in Table 1 and the L9 (3^4) orthogonal array (Jeff and Hamada, 2009).

We used experimental data to determine optimal experimental conditions that we assessed using analysis of variance (ANOVA) (Canovas et al., 2004). Also, an analysis of the signal-to-noise (S/N) ratio was needed to evaluate experimental results. Because the target of this study was to maximize the algae growth rate, *As* accumulation, *As* biotransformation, and *As* release from algal cells, we used the S/N ratio with biggest characteristics as shown in Eq. (1):

$$\frac{S}{N} = -10 \log \left[\frac{\sum_{i=1}^{n} \left(\frac{1}{y_i}\right)^2}{n} \right] \quad (1)$$

where n is the number of repeated measurements under the same experimental conditions, and y_i represents the measured value.

Table 1
Environmental factors of the orthogonal test.

	Factor			
	A NO_3^--N/ (mg/L)	B PO_4^{3-}-P/ (mg/L)	C *pH*	D *As*(V)/(μM)
Level 1	2	0.02	6	0.1
Level 2	4	0.20	8	1.0
Level 3	10	1.00	10	10.0