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

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ABSTRACT: We conducted a series of experiments using Taguchi methods to determine optimum conditions for arsenic (As) biotransformation. We found that N is critical for *M. aeruginosa* As(V) biotransformation, particularly with regard to As(III) transformation. Also, As accumulation benefited from low P levels when combined with high N concentrations. Phosphate was second to As(V) as the primary factor to affect As accumulation. Additionally, we found that the small amounts of As that accumulated under low concentrations of As and high P were tightly stored in living algal cells and were easily released after cell death. Our results will be helpful for the understanding, practical applications, and overall control of the key environmental factors, particularly those associated with algal bioremediation for As-polluted water.

1 INTRODUCTION

Algae are widely distributed in aquatic ecosystems and play an important role in arsenic (As) bioaccumulation and biogeochemical cycling (Duncan *et al.*, 2015; Yan *et al.*, 2016). *Microcystis aeruginosa* is generally tolerant to As(V) and exhibits a stronger As bioaccumulation capacity compared to other freshwater algae (Wang *et al.*, 2017). Many abiotic factors affect the metabolic functions of alga contaminated by As, such as As levels, hydrogen ion levels (pH), and key nutrient concentrations of nitrogen (N) and phosphorus (P) in culture media. To date, very limited information is available on how and to what extent environmental factors influence arsenic (As) biotransformation and release in freshwater algae. 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. Taguchi methods under their relevant statistical assumptions were applied to determine optimum environmental conditions.

2 METHODS

2.1 Experimental design

Three different levels in combination with actual aquatic conditions of each environmental factor were considered (Table 1). Accordingly, we chose an L9 (3⁴) orthogonal array, and we obtained experimental conditions (Table 2) by combining Table 1 and the L9 (3⁴) orthogonal array. We used experimental data to determine optimal experimental conditions and evaluate experimental results, which we assessed using

Table 1. Environmental factors of the orthogonal test.

Factor	NO ₃ ⁻ -N (mg L ⁻¹)	PO ₄ ³⁻ -P (mg L ⁻¹)	pH	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

Table 2. Experimental L₉ (3⁴) orthogonal array.

Treatment	Parameters			
	NO ₃ ⁻ -N (mg L ⁻¹)	PO ₄ ³⁻ -P (mg L ⁻¹)	pH	As(V) μM
E1	2	0.02	6	0.1
E2	2	0.2	8	1.0
E3	2	1.0	10	10
E4	4	0.02	8	10
E5	4	0.2	10	0.1
E6	4	1.0	6	1.0
E7	10	0.02	10	1.0
E8	10	0.2	6	10
E9	10	1.0	8	0.1

analysis of variance (ANOVA) and the signal-to-noise (S/N) ratio with biggest characteristics.

2.2 Batch culture preparation

The *M. aeruginosa* cultures that we used were incubated in BG-11 without adding additional N and P for 48 h after exponential phases of algal suspension growth were centrifuged and washed twice in sterile Milli-Q water. We separated the above cultures into nine equal parts, which were once again centrifuged and washed in sterile Milli-Q water. They were aseptically transferred to nine sterile 1 L Erlenmeyer flasks

Figure 1. Cell density and the specific growth rate of *M. aeruginosa* under different treatments.

Figure 2. Intracellular total As and different As species concentrations in *M. aeruginosa* under different treatments.

(2 replicates per treatment), containing 250 mL of different autoclaved sterilized modified BG-11 media, according to Table 2. After cultured batch treatments in an illuminated incubator shaker for 96 h, We then harvested approximately 20 mL of the algae via centrifugation at $4500 \times g$ for 10 min. Media was also frozen to determine total As (TAs) and the specific As species.

3 RESULTS AND DISCUSSION

3.1 Algae growth

The final cell density and specific growth rate obtained at the conclusion of the 96 h experiment were plotted in Figure 1, which further indicated variation in parameter level combinations. NO_3^- -N was found to be the most influential among the four factors on algal growth while PO_4^{3-} -P was the second most influential factor.

3.2 Intracellular arsenic bioaccumulation

We ascertained TAs content and As species in algal cells after 96 h in culture media (Fig. 2). High As bioaccumulation and its facilitation by N in media indicated that N could affect algae bioremediation in As polluted water. *M. aeruginosa* could accumulate greater As(V) in cells under high As(V) ambient concentrations, coexisting with low P and high N concentrations. Being key factors affecting intracellular TAs and As(V) accumulation, their similarity in chemical properties between P and As determined that P was second to As in rank and order.

3.3 Arsenic biotransformation in media

With the exception of As(V), we detected As biotransformation and DMA in media after 96 h in algal culture (Fig. 3). Although high As(III) and DMA concentrations in media were primarily caused by the high As(V) levels, its reduction to As(III) in media was

Figure 3. Different As species concentrations in culture media under different treatments.

Figure 4. Different As species concentrations in culture media from dead algae cells under different treatments.

inclined to occur in high N and low P concentrations under slightly acidic environments. As the principal factor, N exhibited a significantly positive effect on As(III) concentrations in culture media after As was introduced.

3.4 Arsenic release from dead algae

This study found that As was rapidly released from dead algal cells. Figure 4 shows that approximately 58% to 93% As was released after 8 h resuspension. The high As uptake in algae with increasing initial concentrations of As(V) could result in high As efflux from dead cells. The pH factor yielded principal effects on As(III) and methylated As (OAs) efflux.

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