Water Research 118 (2017) 167–176

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

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

Zhenhong Wang [a, b, c], Zhuanxi Luo [a, c], Changzhou Yan [a, *], Baoshan Xing [c]

[a] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, 361021, China
[b] School of Chemistry and Environment, Fujian Province Key Laboratory of Modern Analytical Science and Separation Technology, Minnan Normal University, Zhangzhou, 363000, China
[c] Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA, 01003, United States

ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form
22 March 2017
Accepted 9 April 2017
Available online 18 April 2017

Keywords:
Arsenic cycling
Dead algae
Bioremediation
Nitrogen
Phosphorus

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

* Corresponding author.
E-mail address: czyan@iue.ac.cn (C. Yan).

http://dx.doi.org/10.1016/j.watres.2017.04.036
0043-1354/

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

Table 2
Experimental L_9 (3^4) orthogonal array.

Treatment	Parameters			
	NO_3^--N/ (mg/L)	PO_4^{3-}-P/ (mg/L)	*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

Based on control factors within the given levels, we adopted the analysis of means (ANOM) statistical approach to develop optimal conditions. In addition to ANOM, we utilized ANOVA to statistically evaluate the influence of each factor on *M. aeruginosa* growth, *As* biotransformation, and release from dead cells in culture media (Zolfaghari et al., 2011). We then calculated the percentage contribution (PC) of each factor using the following equation:

$$PC = \frac{SS_F - (DOF \times V_{Er})}{SS_T} \times 100 \quad (2)$$

where SS_T is the total sum of squares; SS_F is the factorial sum of squares; V_{Er} is the variance of error; and *DOF* is the degree of freedom. ANOVA provided the *DOF*, SS_T, SS_F, and V_{Er} values. We conducted calculations and applications of Taguchi methods as well as univariate ANOVA results using the Minitab 17 software package. We used the Pearson correlation coefficient to reveal relationships between *As* speciation and environmental factors using IBM SPSS Statistics 21 software.

2.2. Phytoplankton culture maintenance

In Milli-Q water (18.2 mΩ cm^{-2}; Millipore), we prepared stock solutions of 1000 mg/L NO_3^--N and *As*(V) as well as 100 mg/L PO_4^{3-}-P from $NaNO_3$, using it as the *N* source, $Na_3AsO_4 \cdot 12H_2O$ (Fluka, p.a.) as the *As*(V) source, and KH_2PO_4 as the *P* source. We also prepared nine modified BG-11 media according to the treatment provided in Table 2. We then adjusted the *pH* solution using 1 M H_2SO_4 or 1 M NaOH.

We obtained *M. aeruginosa* (FACHB-905) cultures from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, China. All *M. aeruginosa* stock cultures were operationally sterile and were prepared using an autoclave sterilized modified BG-11 medium at 25 °C and centrifuged at 90 rpm under a 16:8 light source: dark cycle (40 μmol photons m^{-2} s^{-1}).

2.3. 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 (2 replicates per treatment), containing 250 mL of different autoclaved sterilized modified BG-11 media, according to Table 2. All above treatments were generated in duplicate with a re-suspended initial cell density of approximately 10^6 cells/mL.

Furthermore, we cultured batch treatments in an illuminated incubator shaker for 96 h. We then harvested approximately 20 mL of the algae via centrifugation at 4500 × g for 10 min. We then froze, freeze-dried, and stored cell pellets for analysis. Media was also frozen to determine total *As* (*TAs*) and the specific *As* species. At the same time, to determine changes in specific algal growth rates, we ascertained cell concentrations by measuring optical densities at a wavelength of 682 nm using a Thermo UV–Vis spectrophotometer (Thermo Scientific Evolution 300, Thermo Fisher Scientific, USA) (Wang et al., 2013b).

2.4. Efflux analysis

We quantified *As* efflux from dead algal cells in each treatment after 96 h batch incubation. We first collected algal cells in 50 mL culture media and then successively rinsed them with Milli-Q water and an ice-cold phosphate buffer, after which they were heated in a water bath at 50 °C for 10 min to retrieve dead algal cells (Wang et al., 2014b; Miao and Wang, 2006), which kept cells intact to examine under a microscope. We re-suspended dead algal cells in a 20 mL BG-11 medium for 8 h before they were used to detect *TAs* content in alga and *As* species in media under each treatment.

2.5. Detection and analytical methods

We conducted digestion of the freeze-dried alga to determine cellular content of *TAs* using concentrated nitric acid (HNO_3) (Merck, Darmstadt, Germany), and we used microwaves as the heat source as described by Yin et al. (2011). We determined *TAs* concentrations using an Agilent 7500cx ICP-MS (Agilent Technologies, Santa Clara, CA, USA), operating in collision cell mode. Measured masses were ^{75}As, ^{72}Ge, and ^{103}Rh, the last two serving as checks for signal stability.

Furthermore, *As* species analysis was processed using HPLC-ICP-MS according to our previously described method (Wang et al., 2013b). In brief, we extracted *As* from freeze-dried alga in 1% HNO_3 overnight and digested it using the same microwave accelerated reaction system approach (CEM Microwave Technology Ltd., Matthews, NC, USA). After we filtered the alga through 0.45 μm cellulose acetate syringe filters, extracts as well as the media itself were analyzed for *As* species content. The different *As* species (*As*(III), *As*(V), and organic *As* (OAs) of DMA and MMA) were separated using a PRX-100 anion-exchange column (10 μm; 250 × 4.1 mm; Hamilton, CA, USA) using a precolumn (11.2 mm; from 12 to 20 μm). The mobile phase consisted of 10 mM $NH_4H_2PO_4$ and 10 mM NH_4NO_3, adjusted to a *pH* level of 6.2 using either HNO_3 or ammonia, and run isocratically at 1.0 mL/min. We identified *As* species in samples by comparing their retention time to known standards, including *As*(III), *As*(V), MMA, and DMA, which were prepared using $Na_3AsO_4 \cdot 12H_2O$ (Fluka, p.a.), $NaAsO_2$ (Alfa Aesar), $NaCH_4AsO_3$ (Fluka), and $NaC_2H_6AsO_2$ (Sigma-Aldrich), respectively.

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 Fig. S1, which further indicated variation in parameter level combinations. Cell density ranged from 6.72 to 9.71 × 10^6 cells/mL, with an overall mean of 7.72 ± 1.11 × 10^6 cells/mL and a coefficient of variation (CV) of 0.14; while the specific growth rate ranged from 0.18 to 0.28/d with an overall mean of 0.22 ± 0.04/d and a CV of 0.16. The specific growth rate was similar to bacterioplankton in actual aquatic systems (Rao et al., 2009). Paired with a CV of 0.14 for cell density and 0.16 for the specific growth rate, these results indicated

that variation in *M. aeruginosa* growth affected by environmental factors was relatively high and must therefore not be ignored (Oscar, 2000). We summarized the mean S/N ratio for a given factor at each parameter level for cell density and the specific growth rate as an S/N response, which we provided in Fig. 1a, b (Table S1), respectively. These maximum S/N ratio values represent optimum conditions, yielding the maximum cell density and specific growth rate found in this study (Table S1). Based on the S/N ratio, optimal parameters for algal growth were A (NO_3^--N) at level 3 (10 mg/L), B (PO_4^{3-}-P) at level 2 (0.20 mg/L), C (*pH*) at level 1 (6), and D (*As*(V)) at level 1 (0.1 μM). Combined with the final maximum cell density and specific growth rate at E8 (Fig. S1), optimum levels of NO_3^--N and PO_4^{3-}-P were ascertained at medium *N*: *P* mass ratio of 50. Coincidentally, *M. aeruginosa* was reported to have dynamic increase in the specific growth rate at an optimum medium *N*: *P* ratios between 20 and 100 (Amano et al., 2012; Downing et al., 2005).

Table S2 provides ANOVA results for cell density and the specific growth rate of *M. aeruginosa*, which shows the key effects of factors on algae growth. The four main environmental factors (*As*(V), *N*, *P*, *pH*) had a significant impact on algal growth ($P < 0.05$). According to their magnitudes, the PC rank order of each factor for cell density was: (1) NO_3^--N (42.40%), (2) PO_4^{3-}-P (30.08%), (3) *pH* in culture media (11.29%), and (4) *As*(V) (8.05%). The PC of errors for cell density was 8.63%. For the specific growth rate, the factor rank order was: (1) NO_3^--N (40.72%), (2) PO_4^{3-}-P (28.13%), (3) *As*(V) (9.74%), and (4) *pH* (8.93%). In view of this, 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. Moreover, *N* is extremely important for algae growth since *M. aeruginosa* requires a greater amount of *N* than *P* to support growth, especially under nutrient-limited conditions (Dai et al., 2008). Additionally, the ability of *M. aeruginosa* to uptake *N* is inferior to its ability to uptake *P* (Amano et al., 2012). Specifically, an increase in NO_3^--N could facilitate *M. aeruginosa* growth. Similarly, Lee et al. (2000) showed that *M. aeruginosa* was positively correlated to an increase in nitrate concentrations whether *in situ* or in lab culture experiments. At the same time, *M. aeruginosa* has the capacity to absorb and store *P* as polyphosphate granules in external portions of its cells, which makes it successfully respond to various *P* concentrations while demonstrating no significant changes in growth rate (Otten et al., 2012). Also, its growth was seldom inhibited by *As*(V) under given conditions due to its high *As*(V) tolerance (Wang et al., 2013b). Collectively, *M. aeruginosa* could be used for bioremediation in *As* polluted water, where *N* levels are the key factor in regulating algae growth.

3.2. Intracellular arsenic bioaccumulation

We ascertained *TAs* content and *As* species in algal cells after 96 h in culture media (Fig. S2). The predominant species was *As*(V), accounting for greater than 82% of *TAs*. Also, *As*(III) was found in all treatments, and small amounts of DMA was present in some treatments. This indicated an *As*(V) reduction and methylation in *M. aeruginosa* cells. Pearson correlation coefficient results yielded 0.58 ($P < 0.05$) between intracellular *As*(III) and *N* in media, 0.50 ($P < 0.05$) between *As*(V) and *N*, and −0.48 ($P < 0.05$) between *As*(V) and *P*. Thus, *N* in media could facilitate *As*(V) assimilation as well as its reduction in cells, but *P* in media could inhibit *As*(V) accumulation by impeding *As*(V) influx into cells via *P* transport systems or by competitively binding to active ArsC (an arsenate reductase) sites (Slaughter et al., 2012). In all treatments, the maximum *TAs* content accumulated in alga cells was approximately 412 μg/g (Fig. S2). High *As* bioaccumulation and its facilitation by *N* in media indicated that *N* could affect algae bioremediation in *As* polluted water.

We summarized the mean S/N ratio (Table S3) for a given factor

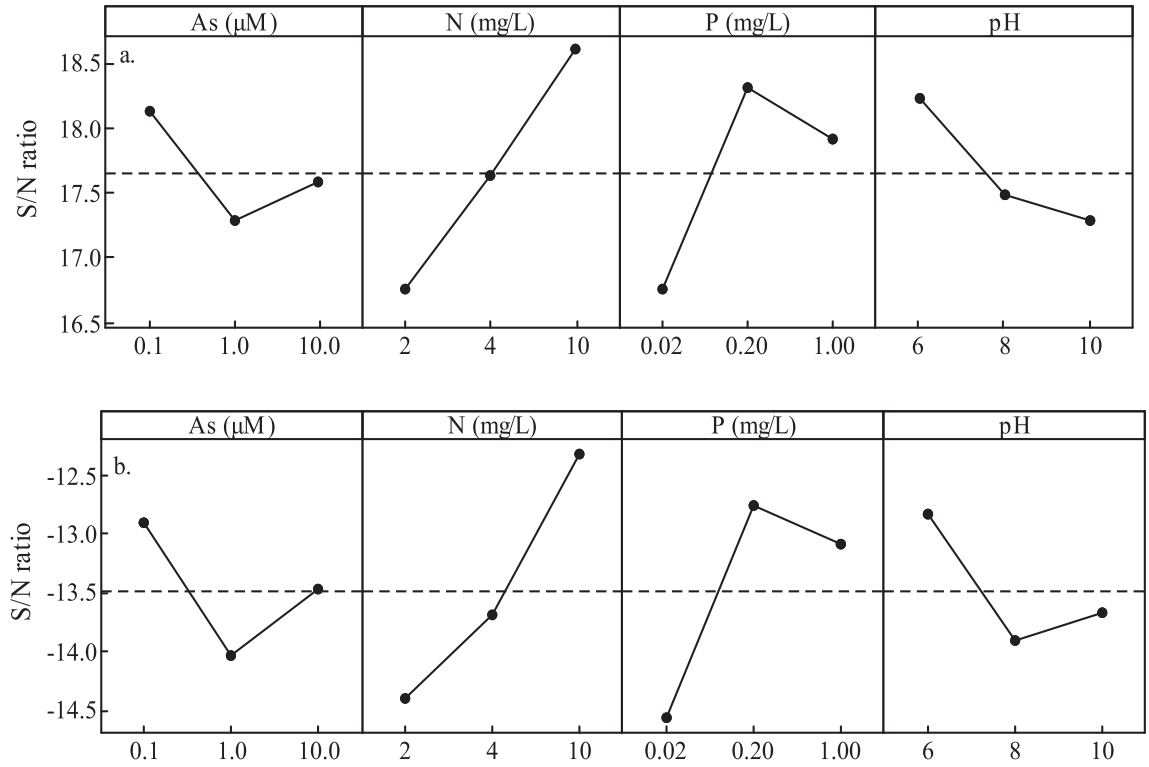

Fig. 1. Mean S/N ratios for (a) cell densities and (b) specific growth rates as affected by *As*, *N*, *P*, and *pH*.

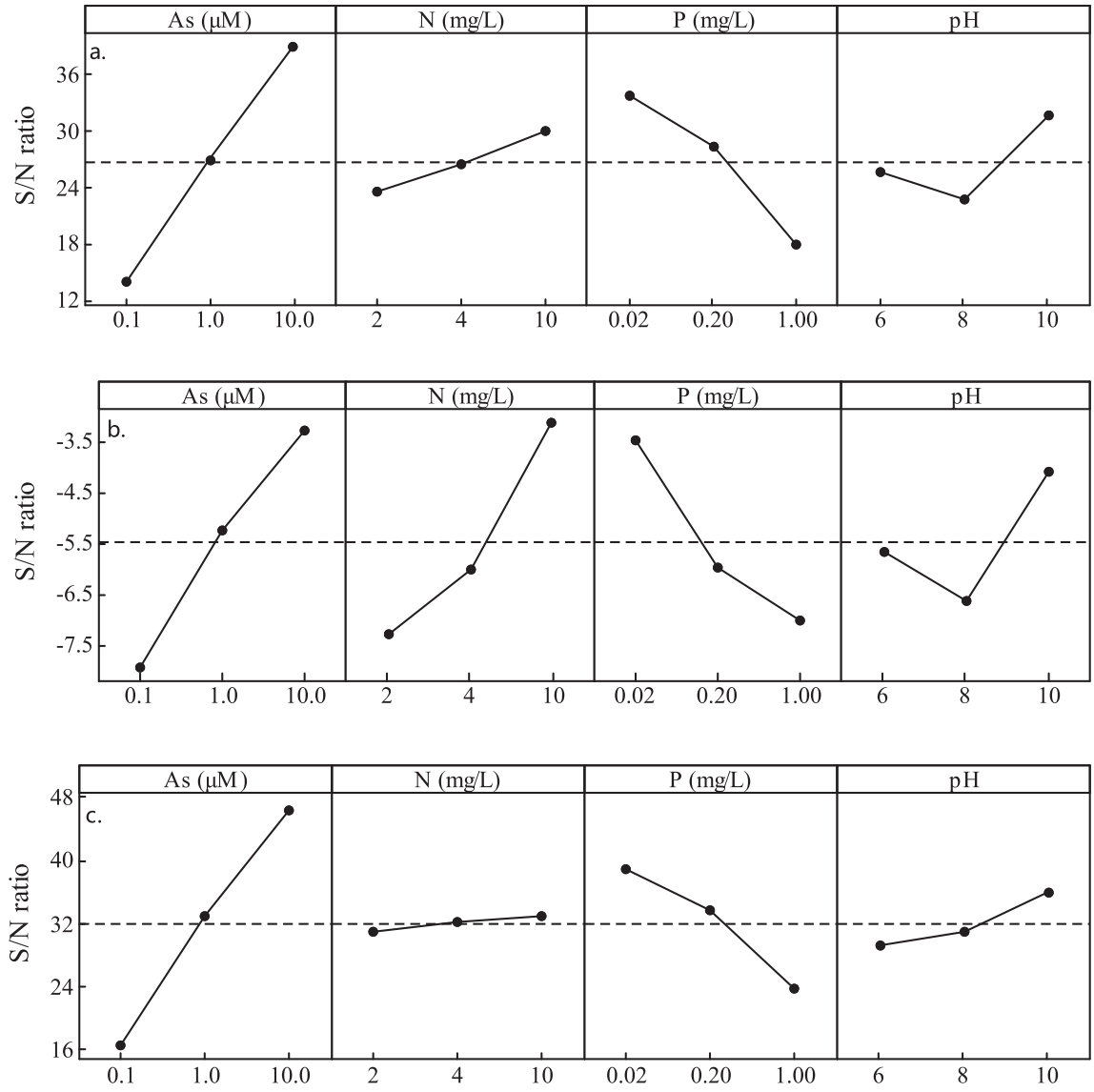

Fig. 2. Mean S/N ratios for (a) intracellular *As*(V), (b) As(III), and (c) *TAs* in *M. aeruginosa* as affected by *As*, *N*, *P*, and *pH*.

at each parameter level for intracellular *As*(V), *As*(III), and *TAs* as an S/N response (Fig. 2a, b, c). Maximum S/N ratio values represent optimum conditions that generated maximum *As* accumulation in this study, and we found them to be under identical conditions for *As*(V), *As*(III), and *TAs*, with A (NO_3^--N) at level 3 (10 mg/L), B (PO_4^{3-}-P) at level 1 (0.02 mg/L), C (*pH*) at level 3 (10), and D (*As*(V)) at level 3 (10.0 μM). This indicated that *M. aeruginosa* could accumulate greater *As*(V) in cells under high *As*(V) ambient concentrations, coexisting with low *P* and high *N* concentrations. Similar studies demonstrated that the addition of *P* in the environment could significantly decrease *As*(V) uptake by *Chlorella salina* (Karadjova et al., 2008) and *M. aeruginosa* (Guo et al., 2011). Although it was suggested that *As* accumulation by *Nostoc* sp. (Maeda et al., 1993) and *Chattonella antiqua* (Yamaoka et al., 1996) also decreased with an *N* increase in media, this effect was opposite to our results by which *As* accumulation increased with the addition of *N*. Moreover, *As* could reduce *N* uptake and adversely affect *N* assimilatory enzymatic activity (Ghosh et al., 2013). Due to its inferior ability in *N* uptake and storage, *M. aeruginosa* growth rates were adversely affected by *N* limitations in media (Amano et al., 2012). These results differed from *Nostoc*, whose superiority under N-limited conditions is due to their N_2 fixation capacity (Havens et al., 2003). Similarly, this also differed from *C. antiqua*, where *As* accumulation was inhibited by *N* at levels higher than 18 mg/L (Yamaoka et al., 1996). These results implied that different algae species respond differently to *As* accumulation in association with changes in *N* concentrations.

Table S4 provides ANOVA results of intracellular *As*(V), *As*(III), and *TAs*. These results illustrated the main effects of environmental factors on intracellular *As* bioaccumulation. The four environmental factors investigated in this study had a significant impact on intracellular *TAs* concentrations ($P < 0.001$), and they were statistically significant for intracellular *As*(V) and *As*(III) ($P < 0.01$) with the exception of factor C (*pH*). Hence, *pH* levels in media had less effect on *As* species transformation and distribution compared to *N*, *P*, and *As*(V) concentrations did in media. Similarly, we found obvious effects of *pH* on specific growth rates of algae in this study, and this was due to the species' good

capacity in *pH* homeostasis (Pang et al., 2013). The PC factor rank order for intracellular *TAs* was: (1) *As*(V) (56.8%), (2) PO_4^{3-}-P (25.5%), (3) NO_3^--N (9.1%), and (4) *pH* in culture media (6.2%). Intracellular *As*(V) yielded similar results with the exception of a close PC value between PO_4^{3-}-P (24.9%) and NO_3^--N (24.8%), illustrating identical effects between *P* and *N*. NO_3^--N (31.4%) was the most influential factor on intracellular *As*(III), followed by *As*(V) (25.2%) and PO_4^{3-}-P (16.2%). 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. Similar findings were reported for *C. reinhardtii*, *Scenedesmus obliquus* (Wang et al., 2013a), *C. salina* (Karadjova et al., 2008), *M. aeruginosa* (Wang et al., 2014b), and *Pennisetum clandestinum* Hochst (Panuccio et al., 2012). Nevertheless, the importance of *N* in influencing intracellular *As*(V) levels, particularly *As*(III), demonstrates that *N* must not be overlooked as an important factor in *As* biogeochemical cycling. Wang et al. also reported the essential importance of *N* in *As* bioaccumulation in *C. reinhardtii* treatment experiments (Wang et al., 2014a). Compared to *As*(V), the higher PC of errors observed for intracellular *As*(III) could originate from its low concentrations. It is important to note that we did not analyze factors associated with DMA since it was detected only in the E4, E7, and E8 treatments, which were coincident with maximum S/N values between the 9 *TAs*, *As*(III), and *As*(V), respectively (Table S3).

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. S3), ranging from 0 to 72.4 μg/L and from 0.52 to 1.79 μg/L, respectively. This detoxification through the biotransformation of *As*(V) to DMA in media further indicated that *M. aeruginosa* could be used to remediate *As* polluted water (Jasrotia et al., 2014). At the same time, we concluded that *As*(III) was the dominant *As* species in culture media, accounting for approximately 81.4% of *TAs* in the E7 treatment. We detected high *As*(III) with only small amounts DMA in culture media for the E4, E7, and E8 treatments, showing that high *As*(V) reduction and *As*(III) efflux with low *As* methylation occurred under conditions of high intracellular *As* accumulation. Results from these treatments further confirmed that although *As*(III) methylation could be the rate-limiting step, *As*(V) reduction could not be (Ye et al., 2012). Compared to other treatments, the higher *As* biotransformation in conjunction with a lower ambient *As*(V) level in the E7 treatment made the higher toxicity of *As*(III) the dominant substitute species for *As*(V), aggravating potential eco-environmental risks. Similar results were observed for *Closterium aciculare* (Hasegawa et al., 2001). This phenomenon must therefore not be disregarded when alga are used in bioremediation for *As* polluted water.

Variations in the S/N ratios for the different environmental factors in culture media are provided in Fig. 3a, b, and c and Table S5 for *As*(V), *As*(III), and DMA. Accordingly, we identified optimum conditions as combinations of A1 (NO_3^--N, 2 mg/L), B3 (PO_4^{3-}-P, 1.00 mg/L), C2 (*pH*, 8), and D3 (*As*(V),10.0 μM) for the highest *As*(V) in culture media (Fig. 3a); A3 (NO_3^--N, 10 mg/L), B1 (PO_4^{3-}-P, 0.02 mg/L), C1 (*pH*, 6), and D3 (As(V), 10.0 μM) for the highest *As*(III) in culture media (Fig. 3b); and A3 (NO_3^--N, 10 mg/L), B3 (PO_4^{3-}-P, 1.00 mg/L), C3 (*pH*, 10), and D3 (*As*(V), 10.0 μM) for the highest DMA in culture media (Fig. 3c). 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 inclined to occur in high *N* and low *P* concentrations under slightly acidic environments. Similarly, low external *P* concentrations could facilitate *As*(V) reduction and excretion (Wang et al., 2014b).

From ANOVA results of *As* biotransformation in culture media (Table S6), we determined that the factor rank order for *As*(V) was: (1) *As*(V) (84.7%), (2) PO_4^{3-}-P (2.8%), (3) NO_3^--N (2.1%), and (4) *pH* in culture media (0.7%). We only found that *As*(V) and PO_4^{3-}-P had significant effects ($P < 0.01$) on *As* biotransformation in culture media. For *As*(III) in culture media, however, the four environmental factors were statistically significant ($P < 0.001$) with a rank order of (1) *As*(V) (47.5%), (2) NO_3^--N (24.1%), (3) PO_4^{3-}-P (19.8%), and (4) *pH* in culture media (6.9%). Although *As*(V) concentrations had the highest PC (47.5%) for DMA in culture media ($P < 0.05$), low concentrations in each treatment could result in a relatively high experimental error (66.5%). Previous studies showed that *As*(III) and DMA excretion could be regulated by *P* levels in culture media (Hellweger et al., 2003). However, as the principal factor, *N* exhibited a significantly positive effect on *As*(III) concentrations in culture media after *As* was introduced (Fig. 3b). In combination with the essential contribution to intracellular *As*(III), *N* should not be overlooked as a factor in *M. aeruginosa As*(V) biotransformation, and this is particularly important for *As*(III) transformation. This would result in algal growth acceleration, which would further improve *As*(III) accumulation and efflux, promoting *As*(V) to *As*(III) reduction.

3.4. Arsenic release from dead algae

This study found that *As* was rapidly released from dead algal cells. Fig. S4 shows that approximately 57.5%–92.9% *As* was released after 8 h resuspension (Fig. S4). Thus, *As* efflux from dead algae could be a potential risk, which rapidly occurs at significant levels after alga blooms in *As* polluted water. We investigated *As*(V), *As*(III), and DMA in efflux media except for the E4, E5, and E9 treatments without *As*(III) being detected (Fig. S5). For treatment E9, organic *As* (OAs) comprised approximately 53.7% of *TAs* in efflux media. For the other treatments, *As*(V) was the main *As* species, ranging from 57.6% to 99.5% of *TAs* efflux in culture media. It should be noted that OAs could be produced as a consequence of the breakdown of dead cells (Hasegawa et al., 2001).

Fig. 4a, b, c, and *d* (Table S7) provide the plot of mean S/N ratios for *As*(V), *As*(III), and DMA and the *As* efflux ratio as a function of the four environmental factors in efflux media. The optimum condition for *As*(V) efflux in media was the combination of A1 (NO_3^--N, 2 mg/L), B2 (PO_4^{3-}-P, 0.20 mg/L), C3 (*pH*, 10), and D3 (*As*(V), 10.0 μM); for *As*(III) it was A2 (NO_3^--N, 4 mg/L), B3 (PO_4^{3-}-P, 1.00 mg/L), C3 (*pH*, 10), and D3 (*As*(V), 10.0 μM); and for DMA it was A2 (NO_3^--N, 4 mg/L), B2 (PO_4^{3-}-P, 0.20 mg/L), C1 (*pH*, 6), and D1 (*As*(V), 0.1 μM). The highest *As* efflux ratios were A2 (NO_3^--N, 4 mg/L), B3 (PO_4^{3-}-P, 1.00 mg/L), C3 (*pH*, 10), and D1 (*As*(V), 0.1 μM). A *pH* level of 10 was optimal for *As* release to actuate in dead alga cells; the exception being DMA at a *pH* level of 6. Likewise, excreted total *As* concentrations from *C. vulgaris* increased with increasing *pH* (Maeda et al., 1992).

From ANOVA results of *As* efflux in media (Table S8), we found that *As*(V) concentrations and *pH* levels were statistically significant for *As*(V) efflux ($P < 0.01$) at approximately 90.2% and 3.8% PCs, respectively, and they displayed an increasing trend as their levels increased (Fig. 4a). The high *As* uptake in algae with increasing initial concentrations of *As*(V) could result in high *As* efflux from dead cells. The factor rank order for *As*(III) efflux was: (1) *pH* in media (35.1%), (2) *As*(V) (32.4%), (3) PO_4^{3-}-P (21.3%), and (4) NO_3^--N (1.02%). The factor rank order for DMA efflux was: (1) PO_4^{3-}-P (46.8%), (2) *pH* in media (20.7%), (3) *As*(V) (16.5%), and (4) NO_3^--N (2.7%). The *pH* factor yielded principal effects on *As*(III) and methylated *As* (OAs) efflux. The minimum *As*(III) efflux concentration occurred at a *pH* level of 8 (Fig. 4b), and methylated *As* in efflux media decreased as *pH* levels increased (Fig. 4c). Similarly, Maeda

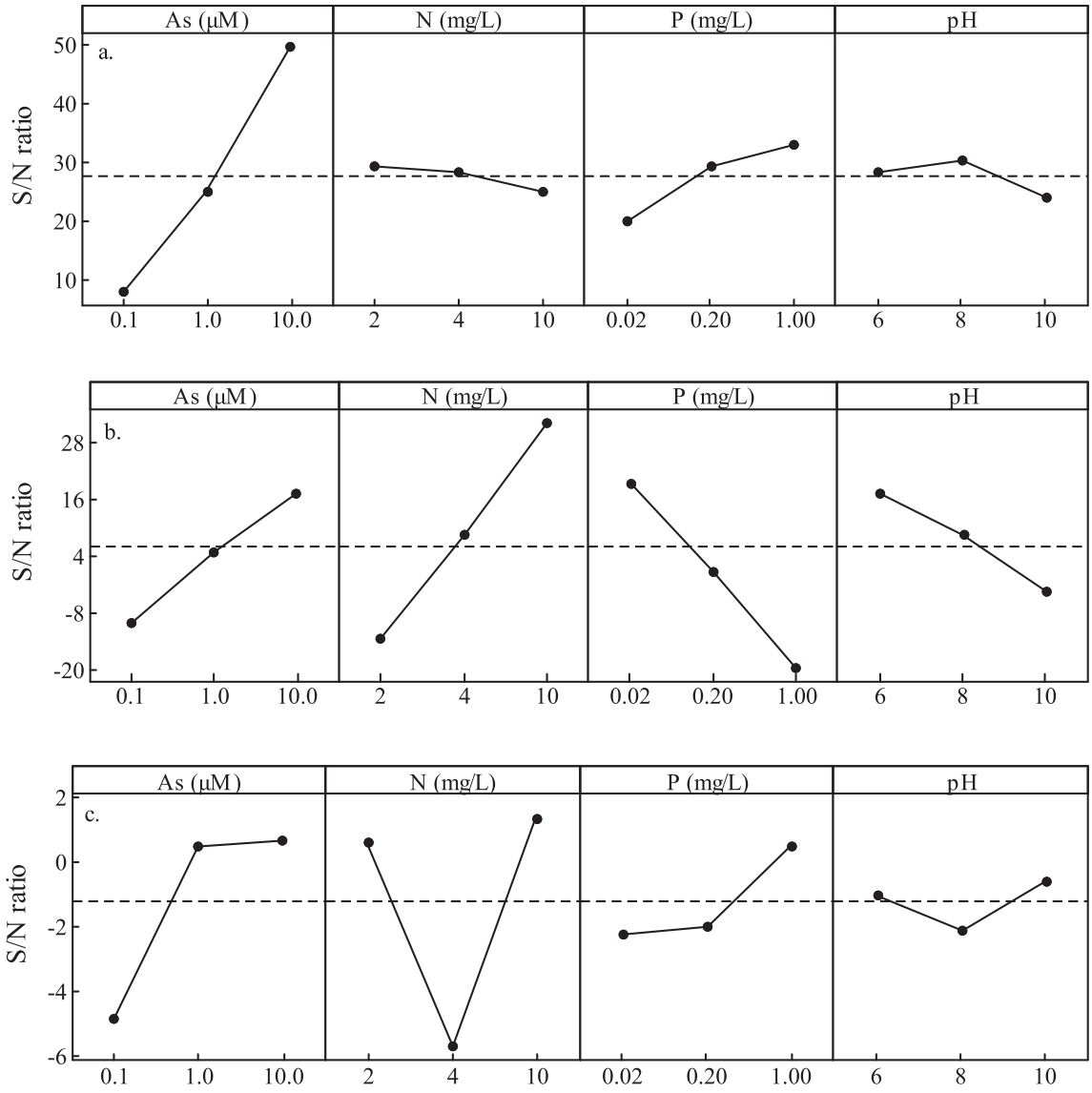

Fig. 3. Mean S/N ratios for (a) *As*(V), (b) *As*(III), and (c) DMA in culture media as affected by *As*, *N*, *P*, and *pH*.

et al. (1992) demonstrated that the relative amount of methylated *As* in excreted *As* increased with decreasing *pH* levels (from 9.0 to 5.3) in *C. vulgaris*. However, reasons for such *pH* effects on *As* excretion are still unclear.

NO_3^--N concentrations exhibited the lowest PCs, and they had no statistical significance on *As* species efflux in media. However, ANOVA results on the *As* efflux ratio from dead algal cells revealed that NO_3^--N concentrations were the key factor after PO_4^{3-}-P concentrations, with PCs of 18.2% and 27.6%, respectively. This highlights the fact that *N* yielded essential effects on *As*(V) biotransformation and biogeochemical cycling. Moreover, *P* had a significant effect on the *As* efflux ratio from alga as well as on DMA in media. We found that optimal *P* concentrations for algal growth was under 0.2 mg/L (Fig. 1), and alga also had the smallest *As* efflux ratio at this *P* concentration (Fig. 4d), which was consistent with DMA efflux results (Fig. 4c). Therefore, small amounts of *As* that accumulated under conditions of low *As* concentrations and high *P* concentrations were securely stored in living algal cells, and they were easily released during decomposition after cell death.

3.5. Environmental implications

By applying *M. aeruginosa* and the Taguchi methods, this study was able to systematically examine the optimal conditions of *As*(V), *pH*, *N*, and *P* that would result in maximal algal cell density, specific growth rates, *As* accumulation and bioaccumulation, and efflux from dead algal cells as well as the principal effects of concentrations of the four controllable environmental factors investigated in this study. Being the first report on how these ambient environmental factors influence *As* metabolism in freshwater algae, this study provides new insight into how ambient environmental factors could be used to regulate *As* metabolism. Our findings will be useful in understanding practical applications and the overall control of key environmental factors, particularly those related to algal bioremediation in *As* polluted water. However, we must also consider in detail the statistical assumptions and limitations involving in no cross term effects while using the Taguchi methods (Parks, 2001).

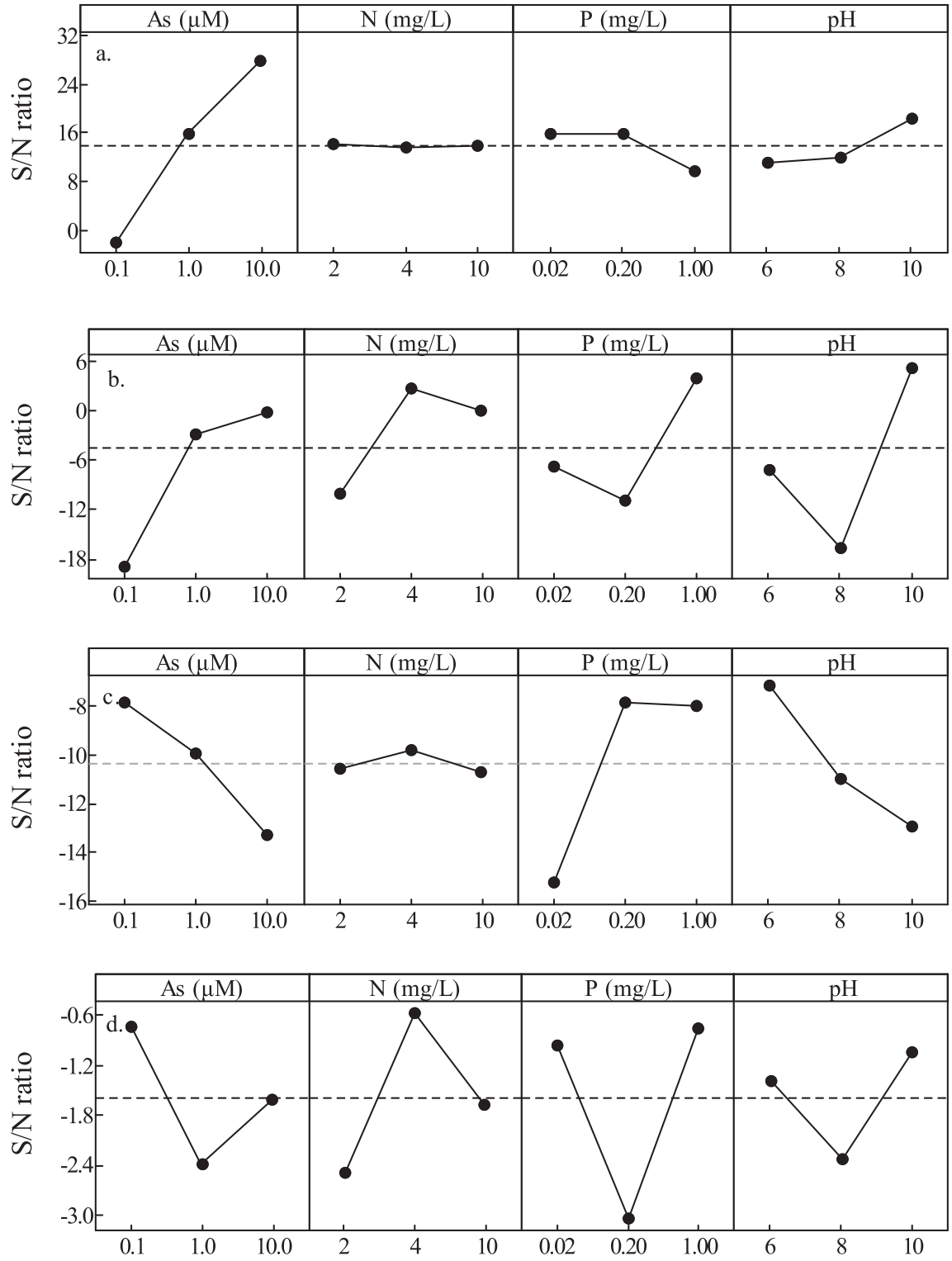

Fig. 4. Mean S/N ratios for (a) *As*(V), (b) *As*(III), (c) DMA, and (d) the *As* efflux ratio in culture media released from dead algal cells as affected by *As*, *N*, *P*, and *pH*.

4. Conclusions

(1) *N* accelerated algal growth, which subsequently improved *As*(III) accumulation and its efflux and in turn promoted *As*(V) to *As*(III) reduction rates. Accordingly, *N* should be treated as a key factor in *M. aeruginosa As*(V) biotransformation, particularly on *As*(III) transformation.

(2) Low *P* and high *N* concentrations in this study were beneficial to *As* accumulation.

Acknowledgments

We would like to thank the anonymous reviewers for their helpful comments. This study was jointly supported by the National Nature Science Foundation of China (project nos. 21277136, 41401552, and 41271484) and the Nature Science Foundation of Fujian Province (2016J01691).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.04.036.

References

Amano, Y., Takahashi, K., Machida, M., 2012. Competition between the cyanobacterium *Microcystis aeruginosa* and the diatom *Cyclotella sp*. under nitrogen-limited condition caused by dilution in eutrophic lake. J. Appl. Phycol. 24 (4), 965–971.

Bahar, M.M., Megharaj, M., Naidu, R., 2013. Toxicity, transformation and accumulation of inorganic arsenic species in a microalga Scenedesmus sp isolated from soil. J. Appl. Phycol. 25 (3), 913–917.

Bears, H., Richards, J.G., Schulte, P.M., 2006. Arsenic exposure alters hepatic arsenic species composition and stress-mediated gene expression in the common killifish (Fundulus heteroclitus). Aquat. Toxicol. 77 (3), 257–266.

Canovas, D., Vooijs, R., Schat, H., de Lorenzo, V., 2004. The role of thiol species in the hypertolerance of *Aspergillus sp* P37 to arsenic. J. Biol. Chem. 279 (49), 51234–51240.

Caumette, G., Koch, I., Estrada, E., Reimer, K.J., 2011. Arsenic speciation in plankton organisms from contaminated lakes: transformations at the base of the freshwater food chain. Environ. Sci. Technol. 45 (23), 9917–9923.

Chen, G.L., Chen, G.B., Li, Y.H., Wu, W.T., 2014a. A study of thermal pyrolysis for castor meal using the Taguchi method. Energy 71, 62–70.

Chen, G.L., Liu, X.M., Xu, J.M., Brookes, P.C., Wu, J.J., 2014b. Arsenic species uptake and subcellular distribution in Vallisneria natans (Lour.) Hara as influenced by aquatic pH. Bull. Environ. Contam. Tox. 92 (4), 478–482.

Dai, R.H., Liu, H.J., Qu, J.H., Zhao, X., Ru, J., Hou, Y.N., 2008. Relationship of energy charge and toxin content of Microcystis aeruginosa in nitrogen-limited or phosphorous-limited cultures. Toxicon 51 (4), 649–658.

Downing, T.G., Sember, C.S., Gehringer, M.M., Leukes, W., 2005. Medium N : P ratios and specific growth rate comodulate microcystin and protein content in *Microcystis aeruginosa* PCC7806 and *M-Aeruginosa* UV027. Microb. Ecol. 49 (3), 468–473.

Duncan, E.G., Maher, W.A., Foster, S.D., 2015. Contribution of arsenic species in unicellular algae to the cycling of arsenic in marine ecosystems. Environ. Sci. Technol. 49 (1), 33–50.

Duncan, E.G., Maher, W.A., Foster, S.D., Krikowa, F., 2013. The influence of arsenate and phosphate exposure on arsenic uptake, metabolism and species formation in the marine phytoplankton Dunaliella tertiolecta. Mar. Chem. 157, 78–85.

Foster, S., Thomson, D., Maher, W., 2008. Uptake and metabolism of arsenate by anexic cultures of the microalgae Dunaliella tertiolecta and Phaeodactylum tricornutum. Mar. Chem. 108 (3–4), 172–183.

Ghosh, S., Saha, J., Biswas, A.K., 2013. Interactive influence of arsenate and selenate on growth and nitrogen metabolism in wheat (*Triticum aestivum L.*) seedlings. Acta Physiol. Plant 35 (6), 1873–1885.

Gong, Y., Chou, H.N., Tu, C.D., Liu, X., Liu, J.T., Song, L.R., 2009. Effects of arsenate on the growth and microcystin production of *Microcystis aeruginosa* isolated from Taiwan as influenced by extracellular phosphate. J. Appl. Phycol. 21 (2), 225–231.

Guo, P., Gong, Y., Wang, C., Liu, X., Liu, J., 2011. Arsenic speciation and effect of arsenate inhibition in a *Microcystis aeruginosa* culture medium under different phosphate regimes. Environ. Toxicol. Chem. 30 (8), 1754–1759.

Hasegawa, H., Rahman, M.A., Kitahara, K., Itaya, Y., Maki, T., Ueda, K., 2010. Seasonal changes of arsenic speciation in lake waters in relation to eutrophication. Sci. Total Environ. 408 (7), 1684–1690.

Hasegawa, H., Sohrin, Y., Seki, K., Sato, M., Norisuye, K., Naito, K., Matsui, M., 2001. Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. Chemosphere 43 (3), 265–272.

Havens, K.E., James, R.T., East, T.L., Smith, V.H., 2003. N : P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. Environ. Pollut. 122 (3), 379–390.

Hellweger, F., Farley, K.J., Lall, U., Di Toro, D.M., 2003. Greedy algae reduce arsenate. Limnol. Oceanogr. 48 (6), 2275–2288.

Jasrotia, S., Kansal, A., Kishore, V.V.N., 2014. Arsenic phyco-remediation by Cladophora algae and measurement of arsenic speciation and location of active absorption site using electron microscopy. Microchem. J. 114, 197–202.

Jeff, W.C., Hamada, M., 2009. Experiments: Planning, Analysis, and Optimisation. J John Wiley and Sons, Inc., Publication.

Karadjova, I.B., Slaveykova, V.I., Tsalev, D.L., 2008. The biouptake and toxicity of arsenic species on the green microalga Chlorella salina in seawater. Aquat. Toxicol. 87 (4), 264–271.

Le, C., Zha, Y., Li, Y., Sun, D., Lu, H., Yin, B., 2010. Eutrophication of lake waters in China: cost, causes, and control. Environ. Manag. 45 (4), 662–668.

Lee, S.J., Jang, M.H., Kim, H.S., Yoon, B.D., Oh, H.M., 2000. Variation of microcystin content of *Microcystis aeruginosa* relative to medium N: P ratio and growth stage. J. Appl. Microbiol. 89 (2), 323–329.

Lei, M., Wan, X.-m., Huang, Z.-c., Chen, T.-b., Li, X.-w., Liu, Y.-r., 2012. First evidence on different transportation modes of arsenic and phosphorus in arsenic hyperaccumulator Pteris vittata. Environ. Pollut. 161, 1–7.

Luo, K.L., Zhang, S.X., Tian, Y., Gao, X., 2014. Arsenic distribution pattern in different sources of drinking water and their geological background in Guanzhong basin, Shaanxi, China. Acta Geol. Sin. Engl. 88 (3), 984–994.

Ma, J., Guo, H., Lei, M., Zhou, X., Li, F., Yu, T., Wei, R., Zhang, H., Zhang, X., Wu, Y., 2015. Arsenic adsorption and its fractions on aquifer sediment: effect of pH, arsenic species, and iron/manganese minerals. Water Air Soil Pollut. 226 (8), 1–15.

Maeda, S., Kusadome, K., Arima, H., Ohki, A., Naka, K., 1992. Biomethylation of arsenic and its excretion by the alga Chlorella-vulgaris. Appl. Organoment. Chem. 6 (4), 407–413.

Maeda, S., Mawatari, K., Ohki, A., Naka, K., 1993. Arsenic metabolism in a freshwater food chain: blue–green alga (Nostoc sp.)→ shrimp (Neocaridina denticulata)→ carp (Cyprinus carpio). Appl. Organomet. Chem. 7 (7), 467–476.

Mahdavi, H., Ulrich, A.C., Liu, Y., 2012. Metal removal from oil sands tailings pond water by indigenous micro-alga. Chemosphere 89 (3), 350–354.

Miao, A.J., Wang, W.X., 2006. Fulfilling iron requirements of a coastal diatom under different temperatures and irradiances. Limnol. Oceanogr. 51 (2), 925–935.

Oscar, T.P., 2000. Variation of lag time and specific growth rate among 11 strains of *Salmonella* inoculated onto sterile ground chicken breast burgers and incubated at 25C. J. Food Saf. 20 (4), 225–236.

Otten, T.G., Xu, H., Qin, B., Zhu, G., Paerl, H.W., 2012. Spatiotemporal patterns and ecophysiology of toxigenic Microcystis blooms in lake Taihu, China: implications for water quality management. Environ. Sci. Technol. 46 (6), 3480–3488.

Pang, C., Zhou, J., Zhang, X., Wu, S., 2013. Experimental study on the influence of pH value, illumination, nutrient, and temperature factors on cyanobacteria growth. In: Intelligent System Design and Engineering Applications (ISDEA), 2013 Third International Conference on. IEEE, pp. 747–750.

Panuccio, M.R., Logoteta, B., Beone, G.M., Cagnin, M., Cacco, G., 2012. Arsenic uptake and speciation and the effects of phosphate nutrition in hydroponically grown kikuyu grass (Pennisetum clandestinum Hochst). Environ. Sci. Pollut. R. 19 (7), 3046–3053.

Parks, J.M., 2001. On stochastic optimization: Taguchi Methods™ demystified; its limitations and fallacy clarified. Probabilist. Eng. Mech. 16 (1), 87–101.

Pfeiffer, M., Batbayar, G., Hofmann, J., Siegfried, K., Karthe, D., Hahn-Tomer, S., 2015. Investigating arsenic (As) occurrence and sources in ground, surface, waste and drinking water in northern Mongolia. Environ. Earth Sci. 73 (2), 649–662.

Rahman, M.A., Hasegawa, H., 2012. Arsenic in freshwater systems: influence of eutrophication on occurrence, distribution, speciation, and bioaccumulation. Appl. Geochem 27 (1), 304–314.

Rao, H.T.S., Yamakanamardi, S.M., Mallaiah, M., 2009. Temporal variation in the specific growth rate of bacterioplankton in the River Cauvery and its four down stream tributaries in Karnataka State, India. Environ. Monit. Assess. 154 (1–4), 169–185.

Rao, R.S., Kumar, C.G., Prakasham, R.S., Hobbs, P.J., 2008. The Taguchi methodology as a statistical tool for biotechnological applications. A Crit. Apprais. Biotechnol. J. 3 (4), 510–523.

Rzymski, P., Niedzielski, P., Karczewski, J., Poniedziałek, B., 2014. Biosorption of toxic metals using freely suspended *Microcystis aeruginosa* biomass. Cent. Eur. J. Chem. 12 (12), 1232–1238.

Sadiq, M., 1997. Arsenic chemistry in soils: an overview of thermodynamic predictions and field observations. Water Air Soil Pollut. 93 (1–4), 117–136.

Slaughter, D.C., Macur, R.E., Inskeep, W.P., 2012. Inhibition of microbial arsenate reduction by phosphate. Microbiol. Res. 167 (3), 151–156.

Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem 17 (5), 517–568.

Sulaymon, A.H., Mohammed, A.A., Al-Musawi, T.J., 2013. Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. Environ. Sci. Pollut. R. 20 (5), 3011–3023.

Sun, F., Yan, Y., Liao, H., Bai, Y., Xing, B., Wu, F., 2014. Biosorption of antimony(V) by freshwater cyanobacteria Microcystis from Lake Taihu, China: effects of pH and competitive ions. Environ. Sci. Pollut. Res. Int. 21 (9), 5836–5848.

Sun, G.F., 2004. Arsenic contamination and arsenicosis in China. Toxicol. Appl. Pharmacol. 198 (3), 268–271.

Wang, N.X., Huang, B., Xu, S., Wei, Z.B., Miao, A.J., Ji, R., Yang, L.Y., 2014a. Effects of nitrogen and phosphorus on arsenite accumulation, oxidation, and toxicity in Chlamydomonas reinhardtii. Aquat. Toxicol. 157, 167–174.

Wang, N.X., Li, Y., Deng, X.H., Miao, A.J., Ji, R., Yang, L.Y., 2013a. Toxicity and bioaccumulation kinetics of arsenate in two freshwater green algae under different phosphate regimes. Water Res. 47 (7), 2497–2506.

Wang, Y., Wang, S., Xu, P., Liu, C., Liu, M., Wang, Y., Wang, C., Zhang, C., Ge, Y., 2015. Review of arsenic speciation, toxicity and metabolism in microalgae. Rev. Environ. Sci. Biotechnol. 14 (3), 427–451.

Wang, Z., Luo, Z., Yan, C., 2013b. Accumulation, transformation, and release of inorganic arsenic by the freshwater cyanobacterium *Microcystis aeruginosa*. Environ. Sci. Pollut. R. 20 (10), 7286–7295.

Wang, Z., Luo, Z., Yan, C., Che, F., Yan, Y., 2014b. Arsenic uptake and depuration

kinetics in *Microcystis aeruginosa* under different phosphate regimes. J. Hazard. Mater. 276, 393–399.
Wurl, O., Zimmer, L., Cutter, G.A., 2013. Arsenic and phosphorus biogeochemistry in the ocean: arsenic species as proxies for P-limitation. Limnol. Oceanogr. 58 (2), 729–740.
Yamaoka, Y., Takimura, O., Fuse, H., Kamimura, K., Murakami, K., 1996. Accumulation of arsenic by rhaphydophyceae Chattonella antiqua (Hada) Ono. Appl. Organomet. Chem. 10 (9), 721–726.
Yan, C., Che, F., Zeng, L., Wang, Z., Du, M., Wei, Q., Wang, Z., Wang, D., Zhen, Z., 2016. Spatial and seasonal changes of arsenic species in Lake Taihu in relation to eutrophication. Sci. Total Environ. 563–564, 496–505.
Ye, J., Rensing, C., Rosen, B.P., Zhu, Y.G., 2012. Arsenic biomethylation by photosynthetic organisms. Trends Plant Sci. 17 (3), 155–162.
Yin, X.-X., Wang, L.H., Bai, R., Huang, H., Sun, G.-X., 2011. Accumulation and transformation of arsenic in the blue-green alga synechocysis sp. PCC6803. Water Air Soil Pollut. 223 (3), 1183–1190.
Zhang, J., Ding, T., Zhang, C., 2013. Biosorption and toxicity responses to arsenite (As [III]) in Scenedesmus quadricauda. Chemosphere 92 (9), 1077–1084.
Zhang, S., Rensing, C., Zhu, Y.G., 2014. Cyanobacteria-mediated arsenic redox dynamics is regulated by phosphate in aquatic environments. Environ. Sci. Technol. 48 (2), 994–1000.
Zolfaghari, G., Esmaili-Sari, A., Anbia, M., Younesi, H., Amirmahmoodi, S., Ghafari-Nazari, A., 2011. Taguchi optimization approach for Pb(II) and Hg(II) removal from aqueous solutions using modified mesoporous carbon. J. Hazard. Mater. 192 (3), 1046–1055.