Journal of Cleaner Production 199 (2018) 112–120

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

Biokinetics of arsenate accumulation and release in *Microcystis aeruginosa* regulated by common environmental factors: Practical implications for enhanced bioremediation

Zhenhong Wang [a, b, e], Zhuanxi Luo [a, *], Changzhou Yan [a, **], Ricki R. Rosenfeldt [c, d], Frank Seitz [c, d], Herong Gui [e]

[a] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[b] College of Chemistry and Environment and Fujian Province Key Laboratory of Modern Analytical Science and Separation Technology, Minnan Normal University, Zhangzhou 363000, China
[c] Institute for Environmental Sciences, University of Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany
[d] nEcoTox, An der Neumuehle 2, 76855 Annweiler, Germany
[e] National Engineering Research Center of Coal Mine Water Hazard Controlling (Suzhou University), Suzhou, Anhui, 234000, China

ARTICLE INFO

Article history:
Received 25 March 2018
Received in revised form
29 May 2018
Accepted 12 July 2018
Available online 18 July 2018

Keywords:
Algal bioremediation
Accumulation
Release
Dead algae
Taguchi

ABSTRACT

Only little information is available on combined effects of abiotic environmental factors on algal arsenate (As^V) metabolic biokinetics. Using the Taguchi statistical method, we investigated four environmental factors including As^V, nitrate (N), orthophosphate (P) and pH for their combined effects on algal growth and arsenic (*As*) uptake but also extracellular adsorption of *Microcystis aeruginosa*, as well as *As* release from dead algal cells. Results showed that an increase of N facilitated *M. aeruginosa* growth and thus was the principal factor for the algal maximum specific growth rate (μ_{max}). P was vital to As^V bioconcentration factor (BCF) and *As* partition coefficients ($LogK_d$) released from deal algal cells. As^V impacted the extracellular *As* adsorption onto the algal cells, which thereby increased with increasing initial As^V level. The initial pH had an imperative effect on the As^V uptake (k_u) and release rate (K_e) from the dead cells. The optimum conditions on As^V metabolic biokinetics were N at 10.0 mg L^{-1} for μ_{max}, P at 0.02 mg L^{-1} for $LogK_d$ and BCF, As^V at 10.0 μM for extracellular *As* adsorption, and pH at 10 for k_u, BCF and extracellular *As* adsorption, but pH at 6 for K_e. Collectively, the condition of low P, high N and alkaline pH level was favorable to *As* accumulation rate of living cells and restrictive to *As* release rate from dead cells of *M. aeruginosa*. The obtained information can pave a road for extensive understanding on efficient utilization of *As* bioremediation of algae in practical environment.

1. Introduction

Humans are exposed to the toxic and carcinogenic substance arsenic (*As*) primarily by their consumption of food and water with an exceedingly varying (wide) concentration (Yan et al., 2015). Arsenic is widely distributed and frequently changes its chemical forms of inorganic *As* and organoarsenicals by many physical-chemical and biological processes in the environment (Miyashita et al., 2016). Arsenate (As^V) as the dominant inorganic *As* chemical species, often occurs its contamination in freshwater at global scale (Hasegawa et al., 2010).

Microalgae are considered as an environmentally-friendly and cost-effective bioremediator for *As*-polluted waters (Bahar et al., 2013; Mahdavi et al., 2012; Sulaymon et al., 2013; Wang et al., 2015). Being a primary producer and the oldest prokaryotic organism in the aquatic environment, cyanobacteria play a critical role in *As* biotransformation and biogeochemical processes (Duncan et al., 2015; Maeda et al., 1990, 1993; Wang et al., 2015; Zhang et al., 2014). They can not only uptake As^V vastly, but also convert it into volatile organic species (Thomas et al., 2004). Especially, *Microcystis aeruginosa* provides a high capacity for *As* accumulation because of its high tolerance towards As^V [5]. Combined with its high tolerance range for changes in pH in the

* Corresponding author.
** Corresponding author.
E-mail addresses: zhhwang1979@163.com (Z. Wang), zxluoire@163.com (Z. Luo), czyan@iue.ac.cn (C. Yan).

https://doi.org/10.1016/j.jclepro.2018.07.131
0959-6526/

surrounding media and its notable potential to accumulate phosphate, *M. aeruginosa* is an optimal microalga for *As* bioremediation.

In the last decade, the bioremediation of metals including *As* as well as their accumulation and uptake dynamics in microalgae have been extensively investigated (Gadd, 1992; Guedes Seixas et al., 2014; Maeda et al., 1993; Pembroke et al., 2015). It showed that some abiotic factors such as nitrogen (N), phosphorus (P) (Hasegawa et al., 2001; Lei et al., 2012; Wang et al., 2014a; Wurl et al., 2013) and pH (Hasegawa et al., 2001; Zhang et al., 2013) can impact the *As* metabolism dynamics within the algal cells. Thereby the factors were investigated either separately (Duncan et al., 2010, 2013) or in combination (Wang et al., 2017) under well controlled conditions in laboratory. P was determined as significant factor influencing the As^V uptake, which contributed largely by P transporters in some organisms (Yan et al., 2017). Arsenate bioaccumulation (kinetics) under different P conditions has been reported for several species (Miao et al., 2012; Panuccio et al., 2012; Wang et al., 2013). Specifically, phosphate limitation and depletion have the potential to induce or improve As^V uptake and corresponding efflux rates of *Chlamydomonas reinhardtii*, *Scenedesmus obliquus* and *M. aeruginosa* (Wang et al., 2013, 2014b). In contrast, an increase of nitrate may decrease the As^V accumulation in *Nostoc sp.* and *Chattonella antiqua* (Maeda et al., 1993; Yamaoka et al., 1996). At the same time, a change in pH can influence the *As* biosorption, toxicity (Ma et al., 2015; Pawlik-Skowronska et al., 2004; Zhang et al., 2013) and biotransformation (Bears et al., 2006; PL and DG, 2002) of microalgae cells in the aquatic environment.

Unfortunately, information regarding combined effects on algal As^V metabolism biokinetics induced by the aforementioned abiotic environmental factors are quite limited (Brinza et al., 2007; Wang et al., 2015). This eventually warranted to further investigation, improving a practical application of algae for *As* bioremediation. Furthermore, little is known about indirect implications as for instance induced by a secondary *As* release into waters after algal death. This may potentially pose different ecological risks (e.g. via settlement and subsequent biomagnification by benthic organisms) for the aquatic environment compared to a primary *As* contamination. To learn about the combined influence of the environmental factors: N, P, pH and the initial As^V level (being applied at ambient levels) on the As^V uptake and release kinetics of *M. aeruginosa*, we investigated the *As* bioaccumulation and efflux dynamics involving algal growth and extracellular and intracellular *As* accumulation as well as *As* release in dead algae. Herein, experimental design of Taguchi method concerned only with the principal effects of selected factors was used in our experiments (Zolfaghari et al., 2011). The percentage contribution effect of each environmental factor on the investigated metabolic biokinetic (*As* bioaccumulation and release) was thus statistically calculated using an analysis of variance (ANOVA) based on Taguchi method. Our new findings offer valuable insights in how to efficiently utilize algae as bioremediation tool to reduce *As* in contaminated water for practical environment.

2. Materials and methods

2.1. Experimental design

The Taguchi method, used to optimize the experimental design, is the same being applied during our previous study (Wang et al., 2017), and can be found described in details in the supportive information (SI Method). Briefly, four common environmental factors including As^V, N, P and pH were considered each at three levels (Table S1) (Yan et al., 2016). The detailed experimental conditions were obtained using a L9 (3^4) orthogonal array (Table S2). Furthermore, we used bigger analysis values of signal-to-noise (S/N) ratio to assess optimal conditions for *As* metabolic biokinetics in *M. aeruginosa*. Additionally, the principal contribution factors for various *As* metabolic biokinetic parameters were evaluated using ANOVA in *M. aeruginosa*. The software of Minitab 17 was used to perform the statistical analysis of the following data obtained.

2.2. M. aeruginosa culture growth

Stock cultures *of M. aeruginosa* (FACHB-905) were maintained in sterilized BG-11 media on shakers at 90 rpm (25 °C) under a 8: 16 h dark-light cycle with a light intensity of 40 μmol photons m^{-2} s^{-1} [21]. We prepared the nine BG-11 media under the Taguchi designed experimental conditions (Table S2). As N, As^V and P source, stock solutions of 1000 mg L^{-1} NO_3^--N from $NaNO_3$ as well as 1000 mg L^{-1} As^V from $Na_3AsO_4{\cdot}12H_2O$ (Fluka, p.a.), and 100 mg L^{-1} PO_4^{3-}-P from KH_2PO_4 were prepared. Additionally, respective pH values were adjusted in the media using 1 M NaOH or 1 M H_2SO_4 at the start of each experiment.

2.3. Arsenic metabolic biokinetics

2.3.1. Uptake experiment and kinetics model

Firstly, *M. aeruginosa* cells were separated into nine equal parts after starving cultures. Then they were aseptically transferred to nine different sterilized BG-11 media with an initial cell density of 10^6 cells mL^{-1} applying the Taguchi designed experimental conditions (Table S2). The batch treatments were cultured in an illuminated incubator which was permanently shaking for 96 h. After 3, 24, 48, 72 and 96 h, approximately 20 mL of the algal solutions were sampled from the exposure flasks to determine total *As* concentrations in the cells. After washing the cells twice with sterile Milli-Q water, the 96 h extracellular adsorbed *As* was then washed off for 10 min using ice-cold phosphate buffer of 1 mM K_2HPO_4, 0.5 mM $Ca(NO_3)_2$ and 5 mM MES. The gained washing buffer was then retained at 4 °C after filtering it through a 0.45 μm syringe filter to determine the extracellular *As* content (Levy et al., 2005). After further 10 min of centrifugation at 4500 × g, the settled algal pellets were freeze-dried for further *As* analysis.

The optical density of algal cells was measured at 682 nm wavelength after 0.5, 3, 24, 48, 72 and 96 h of exposure. The growth kinetics were investigated with the exponential model (P Dalgaard, 2001) shown in Eq. (1):

$$Ln(X_t) = N + \mu_{\max} \times t \tag{1}$$

Where X_t is the optical density (cell mL^{-1}) at time t (d); t is the cultivation time; N is a constant; μ_{max} is the maximum specific growth rate (d^{-1}).

A nonlinear one-compartment model considering a simultaneous *As* uptake and release was used to describe the measured intracellular concentration of *As* in algal cells for each treatment over time according to the following first-order kinetics:

$$[As_{\text{int}}] = k_\mu/k_e \times [As_{med}] \times \left(1 - e^{k_e t}\right) \tag{2}$$

Herein, As_{int} (μg g^{-1} dry weight) is the intracellular concentration of *As* in algal cells; As_{med} (μg L^{-1}) is the *As* concentration in medium assumed to be a constant, and t (h) is the time of *As* exposure; k_u (L g^{-1} h^{-1}) and k_e (h^{-1}) are the *As* uptake and release rate constants for the algae, respectively (Wang et al., 2014b).

Due to the dynamic equilibrium of *As* uptake and release by the algae, the proposed model was only applied if $k_e > 0$. The modeling was performed with the program Graphpad Prism 7.0 (Graphpad Software). The bioconcentration factor of *As* was calculated as BCF

($L\ g^{-1}$) = k_u/k_e (Bradac et al., 2009).

2.3.2. Release experiment and kinetics model

To determine the *As* release rates from dead cells, 50 mL algal solution of each treatment were taken after 96 h incubation and rinsed with ultrapure water and the aforementioned phosphate buffer. To produce dead cells of *M. aeruginosa*, the samples were heated for 10 min at 50 °C using a waterbath (Chen et al., 2003; Miao and Wang, 2006; Wang et al., 2014b). Afterwards the treated algae were resuspended in 20 mL sterilized BG-11 media (same with their initial culture conditions) for 8 h, respectively. At 0.25, 0.5, 0.75, 1, 2, 4, 6, and 8 h, 5-mL aliquots were taken from the solutions to determine the algal total *As* concentrations.

The release rate constant for each treatment were evaluated using a simple first-order kinetic (Eq. (3)) (Bradac et al., 2009).

$$Ke = -1/t \times LnC_t/C_0 \quad (3)$$

Herein, C_0 and C_t represent the intracellular *As* concentration (ng g^{-1}) at the start and time t (h) of release, respectively; K_e is the release rate constant (h^{-1}).

The arsenic partition coefficient ($L\ g^{-1}$) K_d, between the algae and the aqueous phase were calculated using the formula $K_d = Ct/Cw$ (where Cw is the measured concentration of *As* in BG-11 medium; $\mu g\ L^{-1}$) after 8 h elimination.

2.4. Total arsenic analysis

Total arsenic analysis (*TAs*) of algal cells and the media was determined according to our previously reported method (Wang et al., 2014b). Briefly, the freeze dried algae were treated by microwave assisted digestion to measure the total *As* amount (Yin et al., 2011). We measured the *TAs* concentrations using ICP-MS (Agilent 7500cx, U.S.A) (Wang et al., 2014b). The signal stability was checked by the simultaneously measured masses of ^{72}Ge, and ^{103}Rh.

3. Results and discussion

3.1. Kinetics of algal growth

The maximum specific growth rate (μ_{max}) obtained from the experiment is shown in Fig. S1 and was on average as high as 0.22 ± 0.03/d ranging from 0.19 to 0.28/d. The maximum values of the S/N ratios of μ_{max} represented the optimum conditions to develop the maximum algal growth (Fig. 1). Accordingly, the optimal conditions for algal growth were NO_3^--N of 10 mg L^{-1}, PO_4^{3-}-P of 0.20 mg L^{-1}, pH of 6 and As^V of 0.1 μM.

The accomplished ANOVA for analyzing the percentage contribution (PC) of the environmental factors to the μ_{max} of *M. aeruginosa* (Table 1), is shown in the following: NO_3^--N concentrations (44.4%), PO_4^{3-}-P concentration (29.6%), pH (11.1%) and As^V concentration (7.4%). Therefore, NO_3^--N was the most influential factor on algal growth, and PO_4^{3-}-P the second. Also others found P owning a less important role in the growth of *M. aeruginosa* compared to N (Ma et al., 2014). NO_3^--N was found to facilitate *M. aeruginosa* growth with increasing concentrations (Fig. S1). Likewise, Lee et al. observed that *M. aeruginosa* grew increasingly with increasing nitrate concentrations (Lee et al., 2000). P did not significantly change the growth rate of *M. aeruginosa* for the different P concentrations tested, most likely due to the fact that P can be absorbed and stored in the algal cells which made it adapt successfully to different P conditions (Otten et al., 2012; Paerl and Otten, 2013). Compared to N and P, As^V with the lowest contribution compared to other factors indicated its trivial impacts on the growth of *M. aeruginosa*, which can be explained by the high As^V tolerance of *M. aeruginosa* (Wang et al., 2014b).

3.2. Arsenic uptake kinetics and its percentage contribution

3.2.1. Arsenic uptake kinetics

The intracellular *As* concentration increased with increasing culture time for all treatments tested (Fig. S2). According to the R^2 (between 0.79 and 0.97), the proposed nonlinear one-compartment model described the experimental data well (Fig. S3). The estimated uptake rate constant (k_u) for As^V in algal cells for the different conditions ranged from 0.03 ~ 0.35 $L\ g^{-1}\ h^{-1}$, and the efflux rate constants of k_e was from 0.01 to 0.06 h^{-1}. The BCF calculated from k_u/k_e ranged from 0.22 to 20.88 $L\ g^{-1}$ (Fig. S3). The values of k_u were lower than those observed during our previous study, where *M. aeruginosa* was cultured in 10 μM As^V solutions exhibiting k_u values from 6.58 to 136.90 $L\ g^{-1}\ h^{-1}$ (Wang et al., 2014b). Further it was also lower than that reported for the green algae *Chlamydomonas reinhardtii* (0.43–6.06 $L\ g^{-1}\ h^{-1}$) exposed to identical *As* concentrations. However, it was similar to the range of k_u being observed for *Scenedesmus obliquus* (0.05–30.20 $L\ g^{-1}\ h^{-1}$) (Wang et al., 2013). The estimated BCF suggested that the *As* which accumulated in *M. aeruginosa* was significantly different among the different treatment conditions. However, this observation is similar, to what was already concluded for the green algae *Chlamydomonas reinhardtii* (9.6 $L\ g^{-1}$) and *Scenedesmus obliquus* (4.1–17.0 $L\ g^{-1}$) at a 6 d exposure to 10 μM As^V under different P levels (Wang et al., 2013). Together with BCF determinations of other metals such as zinc and cadmium for *M. aeruginosa* ranging from 2.02 ~

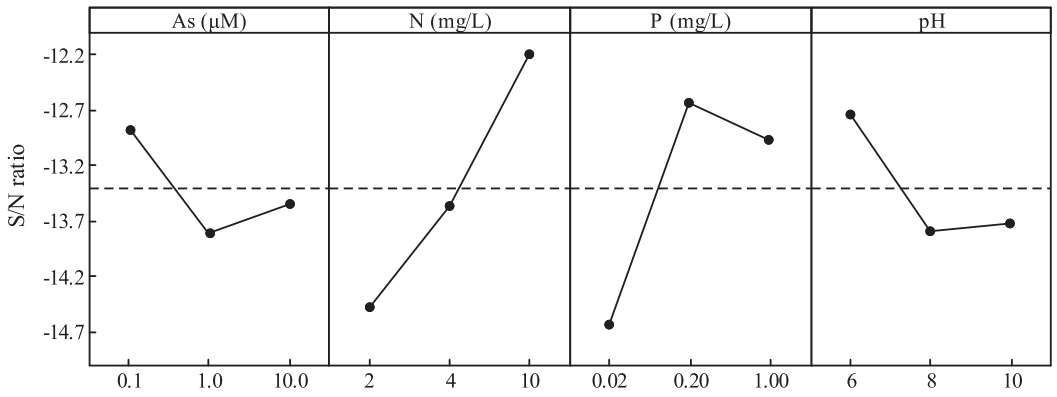

Fig. 1. Mean S/N ratios for the maximum specific growth rates (μ_{max}) as affected by As^V, N, P, and pH.

Table 1
Significance and percentage contribution (PC) of effects of environmental factors (N, P, pH, As^V) on μ_{max} (d^{-1}) based on ANOVA results.

	Variance source	Type III sum of squares	DOF	Mean square	F	*P*	PC
μ_{max}	*N*	0.012	2	0.006	61.762	<0.001	0.444
	P	0.008	2	0.004	39.997	<0.001	0.296
	pH	0.003	2	0.001	14.386	<0.001	0.111
	As^V	0.002	2	0.001	11.149	<0.01	0.074
	V_{Er}	0.002	18	0.000			0.074
	SS_T	1.374	27.000				

101.68 L g^{-1} (Zeng et al., 2012), *M. aeruginosa* strongly implied to be a promising bioremediator candidate to remove heavy metals from natural water. Additionally, the low k_e value indicates a slow static *As* efflux from living algal cells, which was observed to be even smaller after 24 h under P limited (0.16 ± 0.03 h^{-1}) but also P-enriched (1.82 ± 0.67 h^{-1}) conditions (Wang et al., 2014b). This difference was probably due to the changes of environment factors including the main nutrients of N and P for *M. aeruginosa*.

The maximum S/N ratios for k_u and BCF represent optimum conditions inducing the rapidest *As* uptake and the maximum *As* accumulation in *M. aeruginosa*. In contrast, the minimum S/N ratios for k_e show optimum conditions inducing the lowest *As* release from this algae (Fig. 2*a*, *b c*). Specifically, the optimum conditions for the rapidest *As* uptake and maximum *As* accumulation were found to be under identical conditions with NO_3^--N of 4 mg L^{-1} (level 2), PO_4^{3-}-P of 0.02 mg L^{-1} (level 1), pH of 10 (level 3), and As^V of 0.1 μM (level 1) and NO_3^--N of 10 mg L^{-1} (level 3), PO_4^{3-}-P of at 0.02 mg L^{-1} (level 1), pH of 10 (level 3), and As^V of 0.1 μM (level 1) (Fig. 2 a and c), respectively. This indicates that As^V might be accumulated faster and in higher amounts in *M. aeruginosa* under low As^V conditions at low P, high N and alkaline pH levels. In contrast, optimum conditions inducing the lowest *As* release were

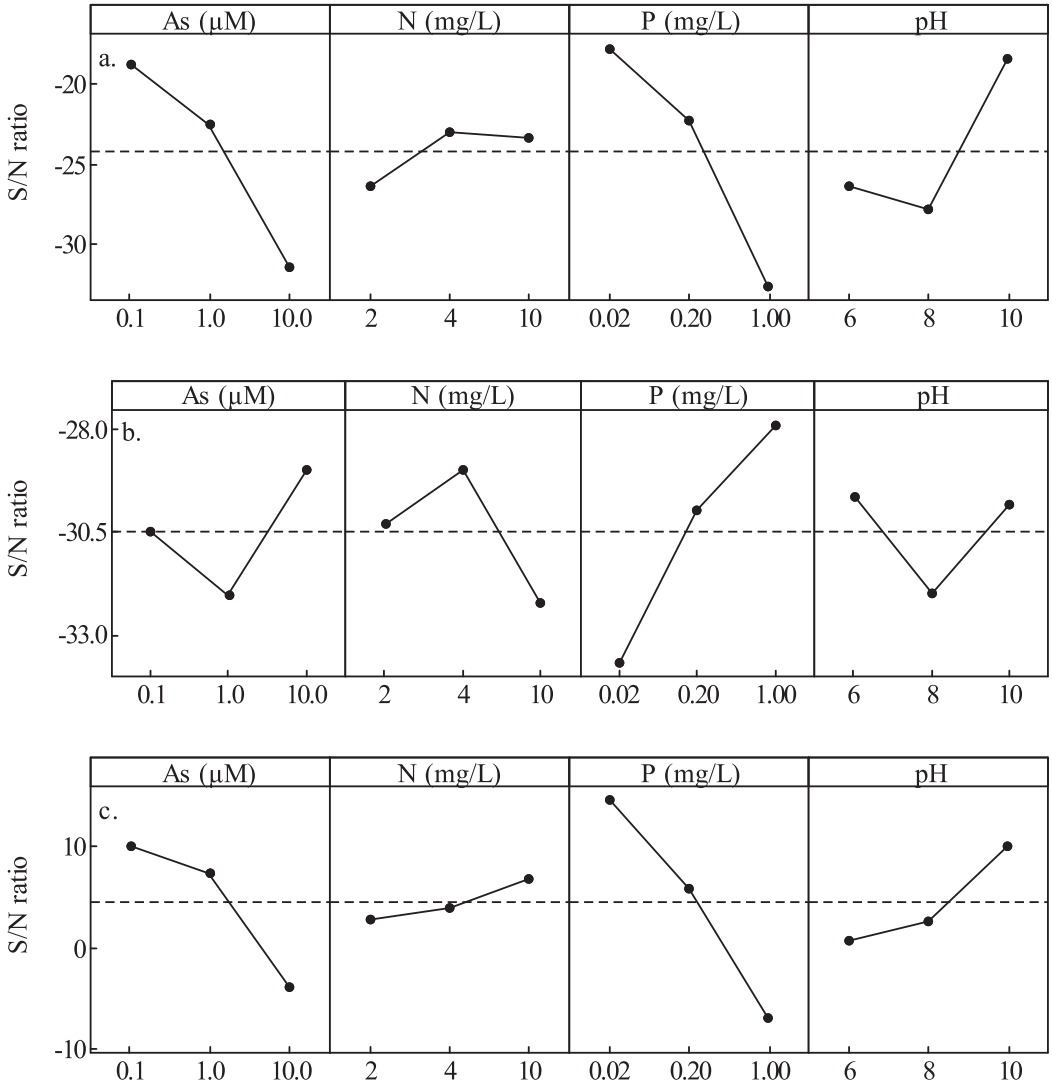

Fig. 2. Mean S/N ratios for *As* uptake and respective dynamic constants: k_u (a), k_e (b) and *BCF* (c) as affected by As^V, N, P, and pH.

identified to exhibit NO_3^--N of 10 mg L^{-1} (level 3), PO_4^{3-}-P of 0.02 mg L^{-1} (level 1), pH of 8 (level 2), and As^V concentration of 1 μM (level 2). This implies that *M. aeruginosa* has a lower efflux of *As* under lower As^V ambient concentration associated with low P but highest N and alkalescent conditions, exhibiting a higher *As* accumulation. Collectively, conditions of lower As^V concentrations together with low P and highest N levels at a high pH are favorable for an *As* accumulation in *M. aeruginosa*. Also, other studies showed that higher P levels decreased the uptake of As^V by *M. aeruginosa* and also *Chlorella salina* (Guo et al., 2011; Karadjova et al., 2008). In contrast, *As* accumulation in *M. aeruginosa* increased with increasing levels of N, which is different for *Nostoc sp.* (Maeda et al., 1993) and *C. antique* (Yamaoka et al., 1996) for which the *As* accumulation lessened with increasing N content.

3.2.2. Percentage contribution

The percentage contribution (PC) rank order for k_u of the tested environmental concentrations in the culture media was found to be: pH (42.5%), PO_4^{3-}-P (24.8%), As^V (22.7%), and NO_3^--N (5.8%) (Table 2). Therefore, the pH value showed a high impact on the *As* uptake kinetics in *M. aeruginosa.* Previous research also observed that pH is the most important factor controlling for cadmium and P uptake in plants (M.B.Kirkham, 2006). One reason is that the *As* biosorption and uptake on and in algal cells is a pH dependent process (Hansen et al., 2006). There the pH value affects the protonation of functional groups carrying polysaccharides and proteins on the algal cell surface as well as the As^V chemistry itself, finally in turn influencing the *As* biosorption and uptake (Gupta and Rastogi, 2008; Tuzun et al., 2005). Similarly, the *As* uptake in *Pteris vittata* L. was shown to be influenced by pH (Tu and Ma, 2003). Furthermore, the optimum pH range for growth of *M. aeruginosa* was demonstrated to be between 8 and 11 (A. Zehnder and P.R.G, 1960). Hence changes of pH in the media can ultimately affect algal growth and its metabolism (Fig. 1), which may additionally impact *As* biosorption and uptake of *M. aeruginosa* [25].

For the BCF, a close PC of PO_4^{3-}-P (28.3%) and pH (27.8%) was observed, indicating the identical effects between P and pH (Table 2). The BCF of *As* decreased with increasing P levels in the media. This antagonistic effect of *As* and P might be caused by transport competition at the same carrier system in *M. aeruginosa.* This was already shown for fungi, bacteria and different plants (Tu and Ma, 2003). The similar chemical properties of P and *As* ascertained that P was the key factor affecting As^V uptake.

Additionally, the higher PC of N (57.3%) for k_e implies that N is also an important parameter for *As* accumulation in *M. aeruginosa* even its $P > 0.05$ (Table 2). This was also shown for the *As* bioaccumulation in *C. reinhardtii* (Wang et al., 2014a). In summary, the PC for *As* accumulation biokinetics in *M. aeruginosa* was: i) alkalescence, ii) low P, lower As^V and highest N concentrations.

3.3. Extracellular As adsorption

Remarkable differences were observed among the nine treatments for the extracellular *As* concentrations ranging from 2.87 to 291.77 μg g^{-1} (Fig. S4). Higher extracellular *As* concentrations were found for the environmental conditions E3, E4 and E8 under high *As* levels in the media (Fig. S4), while they had lower *As* uptake rate constant (Fig. S3) compared with the other treatments. Except for the obviously similar effects of NO_3^--N (A), the optimum media conditions for a maximum extracellular *As* concentration were PO_4^{3-}-P of 0.20 mg L^{-1} (level 2), pH of 10 (level 3), and As^V of 10.0 μM (level 3) in media (Fig. 3). Suggestively, the adsorbed *As* on algal cells was inclined to exist in a high *As* level but lower P level under obvious alkaline environment.

PC results for the extracellular *As* concentration being adsorbed to the algal cells gained from the ANOVA (Table 3) were as high as: 81.5% for As^V, 10.1% for pH, 2.3% for NO_3^--N, and PO_4^{3-}-P (1.5%). Further, As^V and pH showed significant impacts ($P<0.001$) on the extracellular *As* concentration. Thus, as principal factors for a higher extracellular adsorption *of As*, a higher As^V concentration and an alkaline pH value in the media were shown to be favorable (Fig. S4). This is in line with findings showing that a higher *As* adsorption capacity for *Lessonia nigrescens* is pH dependent, however in the case of Hansen et al. the best *As* sorption was found to be at low pH values (Hansen et al., 2006). This difference is potentially caused by the various *As* metabolism of algae under different pH, which is warranted to be further investigated.

3.4. Arsenic release kinetics of dead algae and its percentage contribution

3.4.1. Arsenic release kinetics

A gradual decrease of intracellular *As* concentration was observed for the 8 h release period in dead algal cells (Fig. S5). Additionally, different treatments exhibited significantly different effects on the *As* efflux showing K_e values ranging from 0.11 to 1.45 h^{-1} (Fig. S6). This observation is similar to those being observed for the *As* depuration from living *M. aeruginosa* cells (0.18–0.22 h^{-1})

Table 2
Significance and percentage contribution of effects of environmental factors on k_u (L g^{-1} h^{-1}), *ke* (h^{-1}) and BCF (L g^{-1}) based on ANOVA results.

	Variance source	Type III sum of squares	DOF	Mean square	F	*P*	PC
k_u	*N*	0.028	2	0.014	11.979	<0.001	0.058
	P	0.119	2	0.060	51.516	<0.001	0.248
	pH	0.204	2	0.102	88.334	<0.001	0.425
	As^V	0.109	2	0.055	47.184	<0.001	0.227
	V_{Er}	0.021	18	0.001			0.043
	SS_T	0.856	27				
k_e	*N*	0.001	2	0.000	2.403	>0.05	0.573
	P	0.003	2	0.001	9.057	<0.01	0.099
	pH	0.001	2	0.000	2.990	<0.05	0.096
	As^V	0.000	2	0.000	1.094	>0.05	0.010
	V_{Er}	0.003	18	0.000			0.222
	SS_T	0.041	27				
BCF	*N*	148.803	2	74.401	5.098	<0.05	0.097
	P	431.746	2	215.873	14.793	<0.001	0.283
	pH	424.304	2	212.152	14.538	<0.001	0.278
	As^V	259.998	2	129.999	8.908	<0.01	0.170
	V_{Er}	262.680	18	14.593			0.172
	SS_T	2209.372	27				

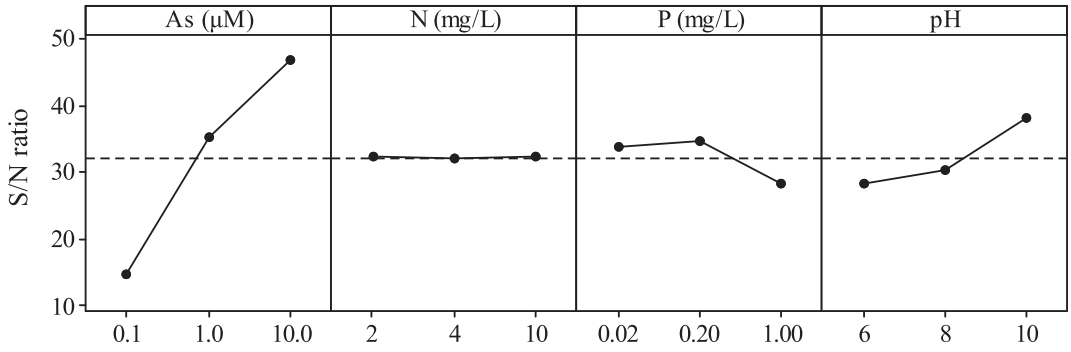

Fig. 3. Mean S/N ratios for extracellular *As* concentrations as affected by As^V, N, P, and pH.

after 24 h As^V exposure applying 10 μM As (Wang et al., 2014b) and also for the As^V accumulation of *C. reinhardtii* (0.12 h^{-1}). However, it was higher than the *As release* of *Scenedesmus obliquus* which was as high as 0.06 h^{-1} (Wang et al., 2013). Particularly, the K_e values in this experiment were less than those observed during our previous experiment showing after 2 h values between 2.94 and 4.80 h^{-1} for dead *M. aeruginosa* cells previously exposed to 10 μM As^V. This however can be attributed to the different P treatments being applied (Wang et al., 2014b). Regardless of the circumstances, the results underline the risk of a potential *As* efflux also for dead cells, which has to be kept in mind when using *M. aeruginosa as As* bioremediation tool.

The Logarithmic partitioning coefficients of *As* ($\log K_d$) varied considerably in our study (3.04–4.17; Fig. S6), being smaller than that of living *M. aeruginosa* for its *As* depuration under P-depleted conditions considering a 10 μM As^V exposure for 24 h ($\log K_d = 4.9$). In line with the maximum value findings for k_e (Fig. S3) and the extracellular *As* concentration (Fig. S4) found in the uptake experiment presented for E3, the calculated K_e for dead algal cells also showed a faster *As* efflux than living cells (Fig. S6). This indicates that conditions of higher arsenate, P and pH but lower N induced a lower As^V uptake and BCF for *M. aeruginosa* but also was favorable for cells dead to release *As* in the surrounding environment. This can be explained by an enhanced extracellular *As* adsorption, which easily desorb from the algal surface after their dead (Fig. S4).

From the S/N ratios for K_e (Fig. 4a), the optimum environmental conditions for K_e were found to be: NO_3^--N of 10 mg L^{-1} (level 3), PO_4^{3-}-P of 0.02 mg L^{-1} (level 1), pH of 6 (level 1), and As^V of 1 μM (level 2), This shows that the release rate of *As* is lower under conditions of higher N and *As* but lower P concentrations and pH values. In contrast, for the $\log K_d$ (Fig. 4b) low concentrations of NO_3^--N (2 mg L^{-1}), PO_4^{3-}-P (0.02 mg L^{-1}) and As^V (1.0 μM) as well as a pH of 8, induced higher *As* contents to retain in the dead cells.

3.4.2. Percentage contribution

The ANOVA results for the *As* release kinetics of K_e and $\log K_d$ (Table 4), showed for *pH*, *N* and *P*, a statistically significant effect ($P<0.001$) and PCs as high as 41.3%, 25.7% and 11.9%. The *pH* had a strong effect on K_e for dead algal cells. Although reasons for the *pH* to affect *As* efflux are still unclear, its critical roles for k_u, BCF and extracellular *As* adsorption, highlight the pH as an important parameter for the *M. aeruginosa* As^V biokinetics. As^V concentration exhibited the lowest PC (5.1%) for K_e indicating that the initial *As* level in the media had only minor effects on the *As* release kinetics for dead algae cells. Additionally, lower K_e values for *M. aeruginosa* were observed for conditions of lower P and higher *As* concentrations. This may be largely due to the good discrimination between P and *As* with a high P/*As* ratio (2012). The *As* efflux rate constant determined in the present study was higher than those of Cd^{2+} and Zn^{2+} (0.029–0.055 h^{-1}) for *M. aeruginosa* (Hajdu et al., 2010). This can be explained by differences in the *As* efflux channels, i.e. anion transporters vs aquaporin channels (Wang et al., 2013). In addition, the efflux rate constant for *As* of *M. aeruginosa* was after a As^V pre-exposure even higher when compared to those observed for *S. obliquus* (0.068–0.11 h^{-1}) and *C. reinhardtii* (0.10–0.12 h^{-1}) under different P conditions (Wang et al., 2013). The higher k_u and BCF for As^V of *M. aeruginosa* under high concentrations of N (10 mg L^{-1}) and low concentrations of P (0.02 mg L^{-1}) (Fig. 2a) had a lower K_e from the dead cells compared to other conditions. This implies a persistent *As* storage capacity and a good *As* bioremediation potentiality of *M. aeruginosa* under conditions of high N and low P concentrations.

The ANOVA further revealed a PC contribution for $\log K_d$ of PO_4^{3-}-P (49.2%) > NO_3^--N (20.7%) > *pH* (11.9%), and As^V (11.2%). Thus, PO_4^{3-}-P, preceded NO_3^--N, was the crucial factor for *As* distribution in algae and aqueous phases ($\log K_d$), which decreased with increasing P concentration. This tendency coincided with the As^V kinetic parameters of k_u and BCF for *M. aeruginosa* and demonstrated that P showed inhibitory effects not only on As^V accumulation but also on *As* efflux from algal cells. In summary, in order to reduce *As* release from dead algal cells (including the release rate but also the *As* amount retained inside), an optimum condition includes higher levels of N but lower concentrations of P and acidic pH values.

3.5. Environmental implications

This systematical investigation of As^V metabolic biokinetics for *M. aeruginosa* using Taguchi method found that P is the most

Table 3
Significance and percentage contribution of effects of environmental factors on extracellular *As* concentrations (μg g^{-1}) based on ANOVA results.

	Variance source	Type III sum of squares	DOF	Mean square	F	*P*	PC
Extracellular *As* concentrations	*N*	6646.778	2	3323.389	4.586	<0.05	0.023
	P	4244.402	2	2122.201	2.928	>0.05	0.015
	pH	28675.392	2	14337.696	19.785	<0.001	0.101
	As^V	232505.464	2	116252.732	160.419	<0.001	0.815
	V_{Er}	13044.273	18	724.682			0.046
	SS_T	285116.310	27				

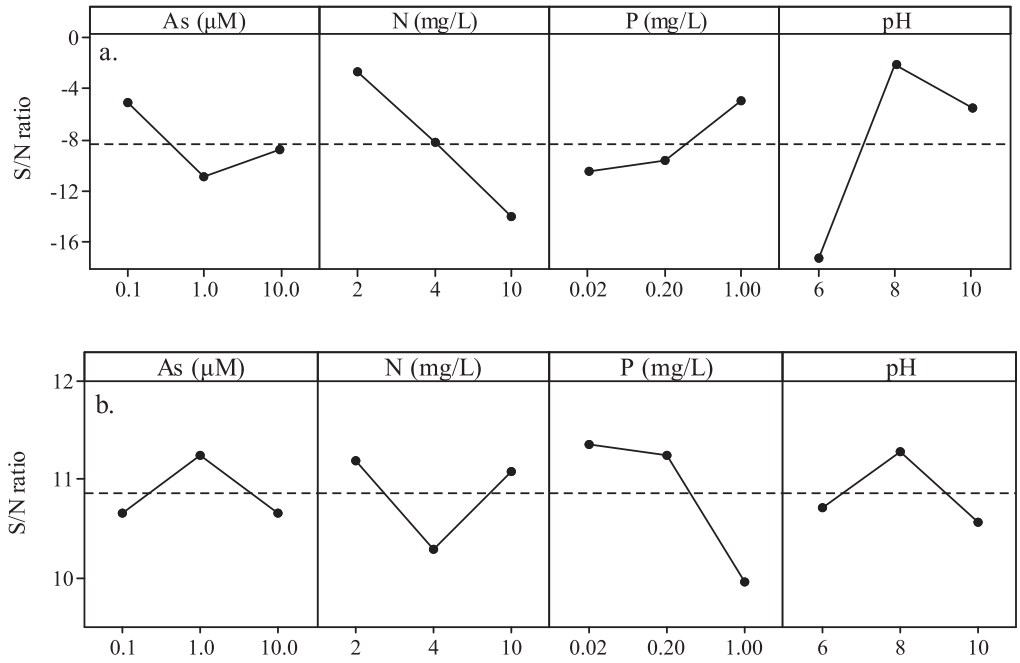

Fig. 4. Mean S/N ratios for *As* efflux rate constant K_e (a) and partition coefficient LogK_d (b) of dead *M. aeruginosa* cells under influence of As^V, N, P, and pH.

Table 4
Significance and percentage contribution of effects of environmental factors on *As* release kinetics of K_e (h^{-1}) and K_d (L g^{-1}) based on ANOVA results.

	Variance source	Type III sum of squares	DOF	Mean square	F	*P*	PC
K_e	*N*	1.377	2	0.688	25.815	<0.001	0.257
	P	1.018	2	0.509	19.085	<0.001	0.190
	pH	2.216	2	1.108	41.543	<0.001	0.413
	As^V	0.272	2	0.136	5.102	<0.05	0.051
	V_{Er}	0.480	18	0.027			0.090
	SS_T	15.723	27				
K_d	*N*	0.721	2	0.360	26.678	<0.001	0.207
	P	1.716	2	0.858	63.521	<0.001	0.492
	pH	0.416	2	0.208	15.396	<0.001	0.119
	As^V	0.392	2	0.196	14.522	<0.001	0.112
	V_{Er}	0.243	18	0.014			0.070
	SS_T	336.483	27				

important factor governing for As^V BCF and LogK_d from dead cells (decreasing with the increasing initial P level). The concentration of N meaningfully affected the algal growth rate (μ_{max}) and the release rate constant (k_e) during the As^V uptake but also K_e of dead algal cells, which showed the maximum μ_{max} and minimum k_e and K_e at its high level of 10.0 mg L^{-1}. As^V only as the main factor affected the extracellular *As* adsorption on algal cells, and showed only minor effects on the algal growth and *As* release kinetics (K_e) and LogK_d (from deal algae). The pH in the media mainly affected the As^V uptake rate (k_u) and release rate (K_e) from dead cells. It also secondly affected the BCF and extracellular adsorption amount of *As*. Additionally, the optimal *pH* value of 6 for *As* release kinetics K_e, and also had the smaller *As* uptake ratio (k_u) and the lowest BCF (Fig. 2 a and c). This shows that a pH of 6 is beneficial to reduce *As* release from dead algae, but unfavorable for *As* accumulation biokinetics. Further, maximum values for k_u, BCF and extracellular *As* adsorption appeared at a pH of 10 together with a lower K_e. This further highlights an optimum pH condition for an As^V bioremediation with *M. aeruginosa* at a pH of 10). After all, a high level of N combined with a relative low level of P at alkaline conditions (pH 10) can meaningfully accelerate an As^V uptake, while diminishing an *As* release. Therefore, these conditions may represent the optimal environmental conditions for an As^V bioremediation with *M. aeruginosa*. This study leads to valuable insights into regulating dynamics of *As* accumulation and release of algae, which favor to practical utilization of algae for *As* bioremediation. Certainly, there are other environmental factors such as temperature, ionic strength and minerals, which would impacted the efficiency of *As* bioremediation. While utilizing the selected algae to remediate contaminated water by *As* in the real environment, it is required to take account to more factors that how they affect dynamics of *As* bioremediation.

Additionally, some *M. aeruginosa'* strains are able to produce microcystins (Pei et al., 2014; Sun et al., 2012), which pose a potential risk to human health. However, they are generally contained in *M. aeruginosa* cells unless the cell membrane is damaged (Teixeira and Rosa, 2007). More studies on this subject are therefore warranted to develop appropriate methods to utilize *M. aeruginosa* as *As* bioremediation without causing cell lysis and microcystins release as well, which guarantee its safety of *As* bioremediation on water environments.

4. Conclusion

(1) P showed to be the most important factor regulating *As* accumulation in living algae cells and efflux levels in dead

cells. Also, N revealed to be a crucial factor impacting algal growth, the release rate during the As^V uptake process but also the *As* efflux rate from dead cells. As^V only as the main factor impacted *As* extracellular adsorption on algal cells, but had minimum effects on algal growth and the *As* release kinetics of deal algae. A pH value of 10 seems optimal for an As^V bioremediation process with *M. aeruginosa, as there* a higher accumulation of *As* in living cell but lower efflux rate from dead cell was observed.

(2) Accordingly, high levels of N combined with low levels of P under alkaline pH conditions can accelerate the As^V uptake and thereby diminish an *As* release, representing optimal conditions for an As^V bioremediation with *M. aeruginosa.*

Acknowledgments

We would like to thank the anonymous reviewers for their helpful comments. This study was jointly supported by Major Science and Technology Program for Water Pollution Control and treatment (2017ZX07101001-02), the National Nature Science Foundation of China (41401552, 41271484 and 41773100) and the Nature Science Foundation of Fujian Province (2016J01691 and 2017Y0081), the New century talent support plan of Minnan Normal University (MX14002).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2018.07.131.

References

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, 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, 257–266.
Bradac, P., Navarro, E., Odzak, N., Behra, R., Sigg, L., 2009. Kinetics of cadmium accumulation in periphyton under freshwater conditions. Environ. Toxicol. Chem. 28, 2108–2116.
Brinza, L., Dring, M.J., Gavrilescu, M., 2007. Marine micro and macro algal species as biosorbents for heavy metals. Environ. Eng. Manag. J. 6, 237–251.
Chen, M., Dei, R.C.H., Wang, W.X., Guo, L.D., 2003. Marine diatom uptake of iron bound with natural colloids of different origins. Mar. Chem. 81, 177–189.
Duncan, E., Foster, S., Maher, W., 2010. Uptake and metabolism of arsenate, methylarsonate and arsenobetaine by axenic cultures of the phytoplankton Dunaliella tertiolecta. Bot. Mar. 53, 377–386.
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.
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, 33–50.
Gadd, G.W.G.A.C.M., 1992. Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuarine green alga ChloreUa salina. Appl. Microbiol. Biotechnol. 37, 270–276.
Guedes Seixas, T., Moreira, I., Siciliano, S., Malm, O., Kehrig, H.A., 2014. Mercury and selenium in tropical marine plankton and their trophic successors. Chemosphere 111, 32–39.
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. SETAC 30, 1754–1759.
Gupta, V.K., Rastogi, A., 2008. Biosorption of lead from aqueous solutions by green algae Spirogyra species: kinetics and equilibrium studies. J. Hazard Mater. 152, 407–414.
Hajdu, R., Pinheiro, J.P., Galceran, J., Slaveykova, V.I., 2010. Modeling of Cd uptake and efflux kinetics in metal-resistant bacterium cupriavidus metallidurans. Environ. Sci. Technol. 44, 4597–4602.
Hansen, H.K., Ribeiro, A., Mateus, E., 2006. Biosorption of arsenic(V) with Lessonia nigrescens. Miner. Eng. 19, 486–490.
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, 265–272.
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, 1684–1690.
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, 264–271.
Kirkham, M.B., 2006. Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. Geoderma 137, 19–32.
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, 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.
Levy, J., Stauber, J.L., Adams, M.S., Maher, W.A., Kirby, J.K., Jolley, D.F., 2005. Toxicity, biotransformation, and mode of action of arsenic in two freshwater microalgae (Chlorella sp. and Monoraphidium arcuatum). Environ. Toxicol. Chem. 24, 2630–2639.
Ma, J., Brookes, J.D., Qin, B., Paerl, H.W., Gao, G., Wu, P., Zhang, W., Deng, J., Zhu, G., Zhang, Y., Xu, H., Niu, H., 2014. Environmental factors controlling colony formation in blooms of the cyanobacteria Microcystis spp. in Lake Taihu, China. Harmful Algae 31, 136–142.
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.
Maeda, S., Ohki, A., Tokuda, T., Ohmine, M., 1990. Transformation of arsenic compounds in a fresh-water food-chain. Appl. Organomet. Chem. 4, 251–254.
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, 467–476.
Mahdavi, H., Ulrich, A.C., Liu, Y., 2012. Metal removal from oil sands tailings pond water by indigenous micro-alga. Chemosphere 89, 350–354.
Miao, A.J., Wang, W.X., 2006. Fulfilling iron requirements of a coastal diatom under different temperatures and irradiances. Limnol. Oceanogr. 51, 925–935.
Miao, A.J., Wang, N.X., Yang, L.Y., Wang, W.X., 2012. Accumulation kinetics of arsenic in Daphnia magna under different phosphorus and food density regimes. Environ. Toxicol. Chem. 31, 1283–1291.
Miyashita, S.-i., Murota, C., Kondo, K., Fujiwara, S., Tsuzuki, M., 2016. Arsenic metabolism in cyanobacteria. Environ. Chem. 13, 577.
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, 3480–3488.
P Dalgaard, K.K., 2001. Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. J. Microbiol. Meth. 43, 183–196.
Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65, 995–1010.
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. Res. 19, 3046–3053.
Pawlik-Skowronska, B., Pirszel, J., Kalinowska, R., Skowronski, T., 2004. Arsenic availability, toxicity and direct role of GSH and phytochelatins in as detoxification in the green alga Stichococcus bacillaris. Aquat. Toxicol. 70, 201–212.
Pei, H.Y., Ma, C.X., Hu, W.R., Sun, F., 2014. The behaviors of Microcystis aeruginosa cells and extracellular microcystins during chitosan flocculation and flocs storage processes. Bioresour. Technol. 151, 314–322.
Pembroke, J.T., Balakrishnan, N., Armshaw, P., 2015. The Potential of the Photoautotroph Synechocystis for Metal Bioremediation.
PL, S., DG, K., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem. 17, 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. Res. 20, 3011–3023.
Sun, F., Pei, H.Y., Hu, W.R., Ma, C.X., 2012. The lysis of Microcystis aeruginosa in AlCl3 coagulation and sedimentation processes. Chem. Eng. J. 193, 196–202.
Teixeira, M.R., Rosa, M.J., 2007. Comparing dissolved air flotation and conventional sedimentation to remove cyanobacterial cells of Microcystis aeruginosa Part II. The effect of water background organics. Separ. Purif. Technol. 53, 126–134.
Thomas, D.J., Waters, S.B., Styblo, M., 2004. Elucidating the pathway for arsenic methylation. Toxicol. Appl. Pharmacol. 198, 319–326.
Tu, S., Ma, L.Q., 2003. Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator Pteris vittata L. under hydroponic conditions. Environ. Exp. Bot. 50, 243–251.
Tuzun, I., Bayramoglu, G., Yalcin, E., Basaran, G., Celik, G., Arica, M.Y., 2005. Equilibrium and kinetic studies on biosorption of Hg(II), Cd(II) and Pb(II) ions onto microalgae Chlamydomonas reinhardtii. J. Environ. Manag. 77, 85–92.
Wang, N.X., Li, Y., Deng, X.H., Miao, A.J., Ji, R., Yang, L.Y., 2013. Toxicity and bioaccumulation kinetics of arsenate in two freshwater green algae under different phosphate regimes. Water Res. 47, 2497–2506.
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, 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.

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, 427–451.
Wang, Z., Luo, Z., Yan, C., Xing, B., 2017. Impacts of environmental factors on arsenate biotransformation and release in Microcystis aeruginosa using the Taguchi experimental design approach. Water Res. 118, 167–176.
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, 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, 721–726.
Yan, Y., Ye, J., Xue, X.M., Zhu, Y.G., 2015. Arsenic demethylation by a C.As lyase in cyanobacterium Nostoc sp. PCC 7120. Environ. Sci. Technol. 49, 14350–14358.
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.
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, 1183–1190.
Yan, Y., Ding, K., Yu, X.-W., Ye, J., Xue, X.-M., 2017. Ability of periplasmic phosphate binding proteins from synechocystis sp. PCC 6803 to discriminate phosphate against arsenate. Water, Air, Soil Pollut. 228.
Zehnder, A., Gorham, P.R., 1960. Factors influencing the growth of microcystis aeruginosa kütz. Emend. Elenkin. Can. J. Microbiol. 6, 645–660.
Zeng, J., Zhao, D.Y., Ji, Y.B., Wu, Q.L., 2012. Comparison of heavy metal accumulation by a bloom-forming cyanobacterium, Microcystis aeruginosa. Chin. Sci. Bull. 57, 3790–3797.
Zhang, J., Ding, T., Zhang, C., 2013. Biosorption and toxicity responses to arsenite (As [III]) in Scenedesmus quadricauda. Chemosphere 92, 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, 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, 1046–1055.