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

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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

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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