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Arsenate biotransformation by *Microcystis aeruginosa* under different nitrogen and phosphorus levels

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ABSTRACT

The arsenate (As(V)) biotransformation by *Microcystis aeruginosa* in a medium with different concentrations of nitrogen (N) and phosphorus (P) has been studied under laboratory conditions. When 15 μg/L As(V) was added, N and P in the medium showed effective regulation on arsenic (As) metabolism in *M. aeruginosa*, resulting in significant differences in the algal growth among different N and P treatments. Under 0.2 mg/L P treatment, increases in N concentration (4–20 mg/L) significantly stimulated the cell growth and therefore indirectly enhanced the production of dimethylarsinic acid (DMA), the main As metabolite, accounting for 71%–79% of the total As in the medium. Meanwhile, 10–20 mg/L N treatments accelerated the ability of As metabolization by *M. aeruginosa*, leading to higher contents of DMA per cell. However, As(V) uptake by *M. aeruginosa* was significantly impeded by 0.5–1.0 mg/L P treatment, resulting in smaller rates of As transformation in *M. aeruginosa* as well as lower contents of As metabolites in the medium. Our data demonstrated that As(V) transformation by *M. aeruginosa* was significantly accelerated by increasing N levels, while it was inhibited by increasing P levels. Overall, both P and N play key roles in As(V) biotransformation processes.

Introduction

Arsenic (As) is a strongly carcinogenic metalloid which is widely distributed in soils, rocks, and natural waters, and has attracted widespread concern due to its severe threat to ecosystem health (Singh et al., 2007). Recently, because of increasing anthropogenic pollution, As contamination in freshwater has gradually become a serious environmental issue in the world (Rahman et al., 2014). The toxicity of As to organisms in freshwater is related to its species and concentration. In general, inorganic As species are more prevalent and more toxic than organoarsenicals (Meharg and Hartley-Whitaker, 2002; Smedley and Kinniburgh, 2002). Arsenate (As(V)) tends to be the dominant species in oxic conditions, while arsenite (As(III)) is predominant in reducing environments (Hasegawa et al., 2010). The dominant organic forms (monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA)) are detected at lower levels in aquatic ecosystems (Akter et al., 2005). It has been shown that conversion of As species primarily depends on the microorganisms in natural ecosystems (Cullen and Reimer, 1989; Levy et al., 2005), thus it is crucial to understand As biotransformation by microorganisms in order to predict its ecological risk in As-contaminated freshwater.

As the important primary producers and prevalent inhabitants of aquatic systems, algae play a vital role in As biogeochemical cycling because they show tolerance toward As and are capable of metabolizing it along several pathways (Levy et al., 2005; Pawlik-Skowronska et al., 2004; Ye et al., 2012; Yin et al., 2012). Interestingly, the ability for As metabolization in algae is related to various environmental factors, including nutrient status, temperature, light intensity, pH, *etc.* (Wang et al., 2015).

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Among these factors, nitrogen (N) and phosphorus (P) are essential elements for various metabolic processes in algal cells, thus the nonnegligible effects of N and P on As biotransformation by algae should be taken into consideration. Previous studies have already manifested that P can effectively affect As toxicity, uptake and efflux, redox processes, and methylation in algae (Guo et al., 2011; Wang et al., 2013a, 2014b; Yan et al., 2014; Zhang et al., 2014). In addition to regulating algal growth, N is also considered an indispensable element to stimulate the synthesis of antioxidants, which can defend against environmental stresses caused by heavy metals (Downing et al., 2005; Liu et al., 2015a; Shao et al., 2009). Therefore, altered N concentration has the potential to affect the tolerance to pollutants in algae. It has been reported that As accumulation in *Nostoc* sp. (Maeda et al., 1993) and *Chlamydomonas reinhardtii* (Wang et al., 2014a) may change under different NO_3^- concentrations in culture. However, to the best of our knowledge, little has been studied regarding the potential influence of N on As biotransformation in algae. Even less is known about the combined influence of N and P on As biotransformation.

Microcystis aeruginosa, as typical freshwater cyanobacteria, are widely distributed in the environment, and are dominant in blue-algal blooms, especially in freshwater with higher trophic status (Oberholster et al., 2004). In view of the coexistence of cyanobacterial blooms and As contamination in many freshwater lakes around the world, it is important to reveal the combined effects of N and P on As biotransformation. In this study, we chose the strain *M. aeruginosa* (FACHB 905) to study the influences of N and P on As biotransformation processes. The contents of different As species both in algae and in culture, as well as the As metabolism pathway in *M. aeruginosa*, were investigated under different concentrations of N and P. Our results can contribute to a better understanding of N and P-regulated interactions between As contaminants and cyanobacteria, and facilitate further understanding of As biogeochemistry in aquatic environments.

1. Materials and methods

1.1. Cultivation and growth condition

The axenic strain of *M. aeruginosa* (FACHB 905), purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, was cultivated in sterile BG11 medium in Erlenmeyer flasks and used throughout the experiment. Sterile precautions were taken with all procedures requiring handling during the experiment to ensure that algal cultures were free from microbial contamination, including ethanol sterilization of the clean bench, open flame sterilization on inoculation, and autoclaved glassware and medium. All the apparatus and the medium used for algal culture and experiments were autoclaved at 121°C for 30 min, and were handled under sterile conditions prior to use. The axenic status of a culture can be confirmed by streaking on an agar plate followed by incubating for 3 days. A bacteria-free algal culture was obtained since no bacteria were observed on the agar algal plate. The axenic *M. aeruginosa* cultures were maintained in a controlled-environment growth chamber under the following conditions: 16:8 light–dark cycle with light intensity of 115 μmol photons/(m^2·sec) at 25°C.

1.2. Experimental design

Cells of *M. aeruginosa* in the exponential growth phase were collected by centrifugation at 9000 r/min at 8°C for 15 min, rinsed with sterile Milli-Q water to remove N and P on the algal surface, and then cultured in sterile BG11 without N and P for 48 hr to consume the remaining N and P *in vivo*. In order to investigate As biotransformation on a practical level in aquatic systems, the selected As(V) concentration (15 μg/L) in this study was similar to those measured in natural aquatic systems under low pollution levels (Smedley and Kinniburgh, 2002; Yan et al., 2016). The effects of N and P were investigated by comparing As(V) metabolization in *M. aeruginosa* cultures, enriched with 15 μg/L As(V) ($Na_3AsO_4{\cdot}12H_2O$, Fluka, p.a.) and specific concentrations of N ($NaNO_3$) and P (K_2HPO_4) as shown in Table 1. Algal cultures without As(V) added were used as control. In addition, an algae-free medium with 15 μg/L As(V) added was prepared to investigate the As abiotic transformation in an axenic environment. The nutrient treatments were selected according to the concentration ranges of N and P in freshwater with different trophic levels and the relationship of N:P ratio reported in previous research (Dos Anjos et al., 2012; Downing and Mccauley, 1992; Paerl et al., 2011; Xie et al., 2003; Xu et al., 2010).

Each experiment was replicated three times and lasted for 8 days. The initial cell density of algal cultures was controlled at 10^6 cells/mL. The flasks were shaken well three times every day and before each sampling. In order to determine As absorption and transformation in *M. aeruginosa*, 10-mL aliquots of algal cultures from each replicate were harvested after exposure to As(V) for 2, 4, 6, and 8 days. Meanwhile, 1.5-mL aliquots of medium were collected each day for the investigation of As excretion into the medium. In addition, the daily cell density of *M. aeruginosa* was determined for the observation of algal cell growth.

1.3. Total As determination

For the determination of intracellular total As (TAs) concentration, the collected algal samples were rinsed with Milli-Q water and ice-cold phosphate buffer (1 mmol/L K_2HPO_4, 5 mmol/L MES, and 0.5 mmol/L $Ca(NO_3)_2$) for 15 min to remove apoplastic As. Then, the freeze-dried algae were kept in 2-mL tubes with 1 mL of concentrated HNO_3 (65%, guaranteed reagent) overnight in preparation for TAs digestion. Briefly, the sample digestion was completed using a microwave accelerated reaction system (MARS-Xpress, CEM Microwave Technology Ltd., USA), with the digestion program as follows: 55°C for 10 min, 75°C for 10 min,

Table 1 – The initial concentrations of nitrogen (N) and phosphorus (P) for five treatments in the test medium.

Experiment	Initial concentration (mg/L)				
	4:0.2	10:0.2	20:0.2	10:0.5	10:1.0
N ($NaNO_3$)	4	10	20	10	10
P (K_2HPO_4)	0.2	0.2	0.2	0.5	1.0
N:P ratio	20	50	100	20	10

and 95°C for 30 min, with a 5 min ramp time between each stage (Wang et al., 2013b; Zhu et al., 2008).

The digestion solutions as well as the medium were filtered through 0.45 μm cellulose acetate membranes, and TAs concentrations in the algae and the medium were determined using inductively coupled plasma mass spectrometry (ICP-MS; Agilent ICP-MS 7500cx, Agilent Technologies, USA).

1.4. Analysis of As speciation

The As speciation in freeze–dried algae was determined by extraction with 1% HNO_3, according to the method described by Wang et al. (2013a). After sample filtration through 0.45 μm cellulose acetate membranes, As species (including As(V), As(III), MMA and DMA) in the algae extracts as well as the medium were determined by high performance liquid chromatography (HPLC; Agilent LC1100 series, Agilent Technologies, USA) coupled with ICP-MS using an anion-exchange column as detailed by Zhu et al. (2008). Chromatographic columns included a precolumn (11.2 mm, 12–20 μm) and a PRP-X100 10 μm anion exchange column (250 × 4.1 mm) purchased from Hamilton. Arsenic species were separated with a mobile phase of 10 mmol/L $NH_4H_2PO_4$ and 10 mmol/L NH_4NO_3 (regulated to pH 6.2 using ultrapure nitric acid or ammonia), and flowed isocratically through the column at 1.0 mL/min with a peristaltic pump. ^{72}Ge and ^{103}Rh were chosen as internal standards to ensure signal correction and stability. The As species in samples were identified by comparing their retention times to known standards, and their contents were calculated based on peak areas. The total concentrations of As species (As(V), As(III), MMA and DMA) accounted for 90%–99% of TAs concentrations.

1.5. Quality control and data analysis

For quality control, a standard for As species was measured every 15 samples for analytical accuracy. The detection limits of HPLC-ICP-MS were 0.07, 0.07, 0.12 and 0.20 μg/L for As(III), DMA, MMA and As(V), respectively (three times the standard deviation of the blank). The precisions (relative standard deviation) of five replicate determinations were 3.5%, 4.5% and 2.4% for inorganic As, MMA, and DMA at 0.2 μg/L, respectively. Meanwhile, standard reference material Bush branches and leaves (GBW07603) were used to check the accuracy of the analyses. The measured values for As (1.17 ± 0.04 mg/kg) were similar to the certified values (1.25 ± 0.15 mg/kg, $n = 3$), with the recovery of 91%–96%.

SPSS 18.0 and Origin 8.5 software were used for statistical analyses and figure preparation, respectively. The significant differences of the treatment effects were assessed by analysis of variance (ANOVA) with post-hoc multiple comparisons (Tukey's test). A significance level of $p < 0.05$ was accepted for all statistical analyses.

The growth rate of the algae (R_a, cells/mL/day) was calculated using Eq. (1):

$$R_a = (\ln C_t - \ln C_0)/t \quad (1)$$

C_0 (cells/mL) and C_t (cells/mL) are the initial cell density and the cell density at culture period t (day), respectively.

The release rate of As metabolites (As(III), MMA and DMA) (R_{As}, μmol/g/day) from the algal cells into the medium was calculated using Eq. (2):

$$R_{As} = ((C_{ex} \times V)/DW_a) \times (1/t) \quad (2)$$

C_{ex} (μmol/L) represents the As concentration in the medium; V (L) is the volume of the medium; DW_a (g) represents the algal dry weight; t (day) represents the culture period.

The content of As uptake per cell of algae ($C_{\text{As uptake}}$, fg/cell) was calculated according to Liu et al. (2015b) and Baker and Wallschläger (2016) with Eq. (3):

$$C_{\text{As uptake}} = (C_{in} + C_{ex})/N \quad (3)$$

C_{in} (μg/L) represents the concentration of intracellular As species (including As(V), As(III), MMA and DMA). C_{ex} (μg/L) represents the concentrations of As species in the medium (including As(III), MMA and DMA). N (10^6 cells/mL) was the cell density of *M. aeruginosa*.

2. Results

2.1. The growth of *M. aeruginosa* under different N and P concentrations

In the control groups, no significant differences were observed in cell growth of *M. aeruginosa* among the five N and P conditions from days 1 to 6; the algal growth under 4 mg/L N and 0.2 mg/L P treatment was obviously ($p < 0.01$) lower than the other treatments on days 7–8 (Fig. 1a, Appendix A Table S1). However, when As(V) was present, cell growth of *M. aeruginosa* exhibited significant differences ($p < 0.05$) among these nutrient treatments within days 1–8 (Fig. 1b, Appendix A Table S1). Specifically, under the condition of 0.2 mg/L P, the algal growth was significantly accelerated at 10–20 mg/L N, compared to that at 4 mg/L N; under the condition of 10 mg/L N, the algal growth rate was significantly elevated from 0.165 ± 0.002 day^{-1} to 0.181 ± 0.002 day^{-1} with increasing P concentration. In addition, the final biomass and chlorophyll-*a* contents of *M. aeruginosa* (day 8) showed similar differences among the five nutrient treatments, with the maximum values achieved at 10 mg/L N and 1.0 mg/L P treatment (Appendix A Fig. S1). Notably, under the lowest N and P conditions, the algal growth exhibited obvious ($p < 0.05$) slowdown from the fourth day to the eighth day, and the slowest growth rate was in accordance with the lowest biomass and chlorophyll-*a* contents (Appendix A Fig. S1).

2.2. Arsenate absorption and transformation in *M. aeruginosa* under different N and P concentrations

As shown in Fig. 2, the TAs concentration in *M. aeruginosa* exhibited a decreasing tendency throughout the 8-day incubation period, with As(V) being the predominant As species, accounting for 59%–94% of TAs; meanwhile, small amounts of As(III) (5%–26%), MMA (<4%) and DMA (0–15%) were also detected in *M. aeruginosa*. In addition, during the 8-day incubation, As(V) absorption and transformation were significantly ($p < 0.05$) different among the five N and P conditions (Fig. 2, Appendix A Table S2).

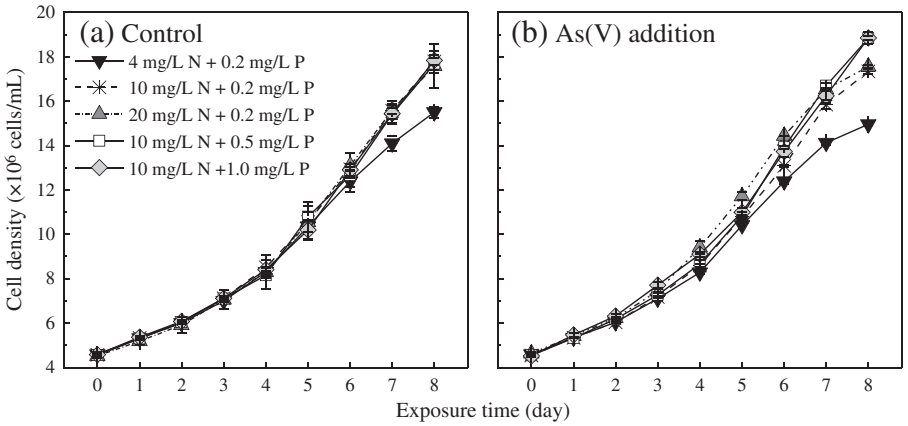

Fig. 1 – Growth of *M. aeruginosa* in control (a) and arsenate (As(V)) addition (b) treatments under different nitrogen (N) and phosphorus (P) concentrations during the 8 days cultivation. Data are means ± standard deviation (n = 3).

Under the 0.2 mg/L P treatment (Fig. 2a–c), As(V) contents in *M. aeruginosa* at 10–20 mg/L N were obviously ($p < 0.05$) lower than at 4 mg/L N on day 2. However, the results were reversed in the concentrations of intracellular As(III). Furthermore, MMA and DMA (which appeared on days 6–8 and days 2–8, respectively) showed significantly higher levels under 10–20 mg/L N treatment. Besides, during the incubation period, the contents of intracellular As(III) and DMA increased firstly, and subsequently showed continuous decline, exhibiting the production of As metabolites and their excretion from the algae.

Under the 10 mg/L N treatment (Fig. 2b, d, and e), intracellular As(V) content rapidly decreased staring on the fourth day at 0.2 mg/L P, compared to that at 0.5–1.0 mg/L P. Correspondingly, intracellular DMA occurred earlier (day 2) at 0.2 mg/L P than that at 0.5–1.0 mg/L P (day 4 and day 6, respectively). Moreover, after the 8-day treatment, the DMA concentration at 0.2 mg/L P treatment was 1.36- and 2.29-fold higher than that at 0.5 mg/L and 1.0 mg/L P treatment, respectively, exhibiting a negative correlation between P level and the production of DMA. A similar result was observed in the intracellular MMA concentration. Notably, MMA was not detected in *M. aeruginosa* at the 1.0 mg/L P treatment level throughout the incubation period.

2.3. *Arsenic excretion by* ***M. aeruginosa*** *under different N and P concentrations*

Fig. 3 shows the decrease in As(V) concentration and production of multiple As metabolites throughout the whole incubation period. Because no transformation of As(V) was observed in algae-free medium (data not shown), it could be confirmed that

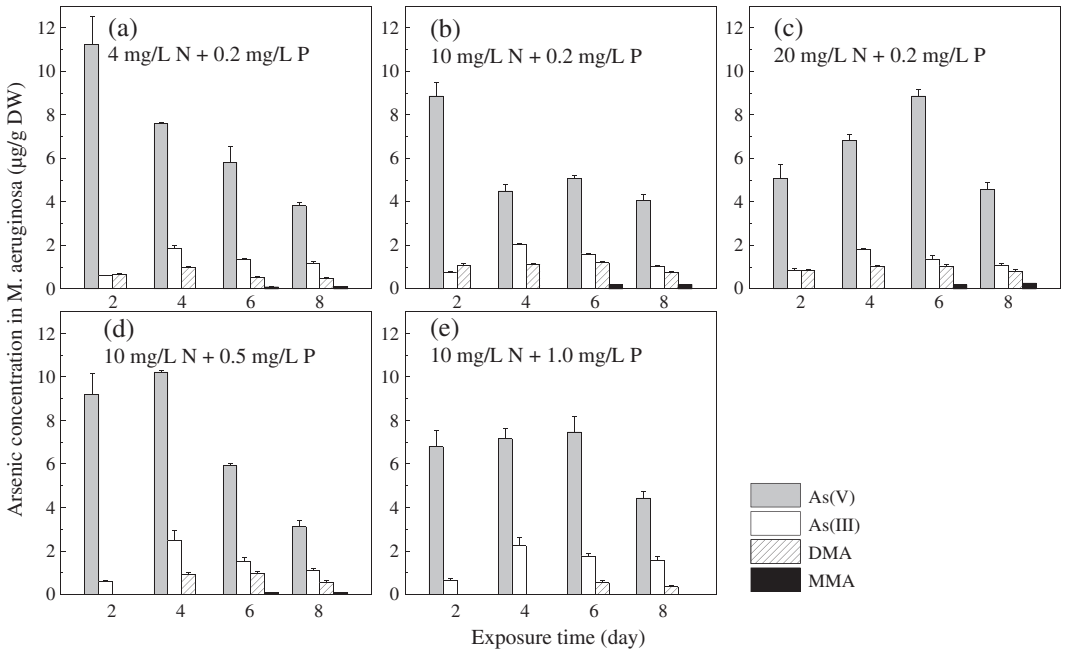

Fig. 2 – Arsenic species in *M. aeruginosa* after exposure to 15 μg/L As(V) for days 2, 4, 6, and 8 under five treatments. Data are means ± standard deviation (n = 3).

As reduction and methylation occurred due to the presence of *M. aeruginosa*. Particularly, significant differences were observed in the contents of As species in the medium under different N and P treatments (Appendix A Table S3).

When the *M. aeruginosa* was treated with 15 μg/L As(V) and 0.2 mg/L P (Fig. 3a–c), the As(V) concentration on the first day quickly declined to 7.03, 7.27 and 6.71 μg/L at 4, 10, and 20 mg/L N treatments, respectively. Meanwhile, as the main metabolite, DMA gradually became the predominant As species in the medium. Small amounts of As(III) (at levels of 1.2%–5.6%) and MMA (1.9%–13.5%) were also found in the 1st–2nd days and 3rd–8th days, respectively. Furthermore, the statistical results indicated that DMA and MMA concentrations were significantly ($p < 0.01$) positively correlated with the levels of N; after 8 days of incubation, the content of DMA was stimulated by up to 71%, 74%, and 79% of TAs with increasing N treatment (from 4 to 20 mg/L), respectively.

As seen in Fig. 3b, d and e, when the *M. aeruginosa* was treated with 15 μg/L As(V) and 10 mg/L N, the As(V) concentration under 0.2 mg/L P treatment rapidly decreased to 1.40 μg/L within 8 days, compared to a significantly slower decline ($p < 0.01$) of As(V) concentration under 0.5–1.0 mg/L P treatment. Furthermore, As(III) was affected by 0.5–1.0 mg/L P treatment and was not detected in the whole incubation period. Notably, the concentrations of MMA and DMA showed significantly negative correlations ($p < 0.01$) with the P levels in the medium, irrespective of the N levels. In particular, DMA content was inhibited by up to 66% and 50% of TAs on day 8 with 0.5 and 1.0 mg/L P treatments, respectively.

Obvious differences in the release rates of the As metabolites were also observed under different N and P treatments (Fig. 4). Under the condition of 0.2 mg/L P, the As(III) average release rate at 20 mg/L N was lower than that at 4 and 10 mg/L N, but the average release rates of MMA and DMA were significantly ($p < 0.01$) enhanced with increasing N concentrations, which was similar to effective acceleration of As methylation by N. Under the condition of 10 mg/L N, however, increases in the average release rates of MMA and DMA significantly ($p < 0.01$) correlated with decreases in P levels, with the maximum values of 0.10 and 0.83 μmol/g/day, respectively, under 0.2 mg/L P treatment.

2.4. Fate of As(V) in *M. aeruginosa* cells under different nutrient treatments

Using the As uptake (sum of intracellular As and excreted As) and cell culture counts, a difference in As(V) metabolism pathway in the *M. aeruginosa* was observed among different nutrient treatments. After an 8-day incubation, the content of As uptake was obviously enhanced under 0.2 mg/L P treatment (>0.7 fg/cell), irrespective of the N levels. Furthermore, although As(V) accumulation dominated in the cellular process, increased intracellular organic As (sum of MMA and DMA at about 9%) and DMA excretion (approximately 39%) were observed at higher levels of N treatment (10–20 mg/L) (Figs. 5a and b, 6a and b). However, a low level of As uptake (<0.7 fg/cell) was observed within cultures of *M. aeruginosa* exposed to 15 μg/L As(V) with higher P concentration (0.5–1.0 mg/L); meanwhile, the occurrence of biologically-produced methylarsenicals was slight at the highest P level, and the vast majority of intracellular As species remained as inorganic As, accounting for 76% of the As uptake (Figs. 5d, 6d).

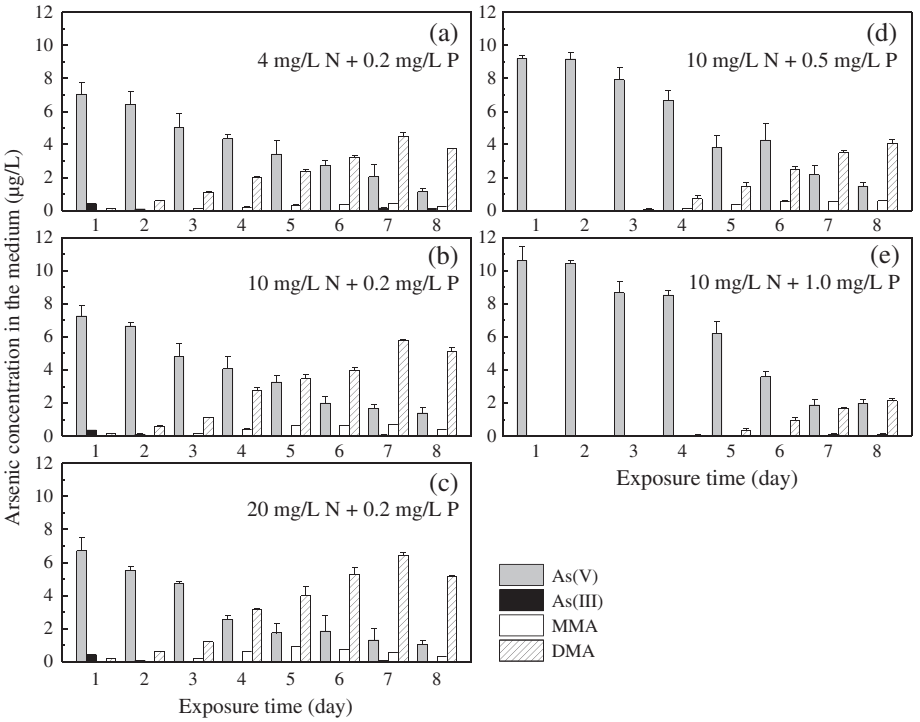

Fig. 3 – Arsenic species in the medium after exposure to 15 μg/L As(V) for days 1–8 under five treatments. Data are means ± standard deviation ($n = 3$).

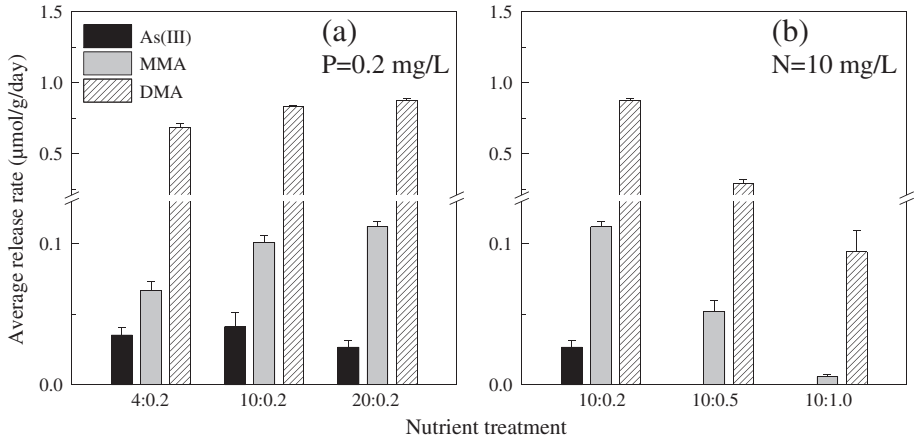

Fig. 4 – The average release rates of different As species into the medium under 0.2 mg/L P (a) and 10 mg/L N (b) conditions after As(V) exposure. Data are means ± standard deviation (*n* = 3).

3. Discussion

The coexistence of As contamination, nutrient excess and algal bloom occurs commonly in freshwater (Barringer et al., 2011; Liu et al., 2014; Yan et al., 2016; Zhang et al., 2013). However, although the interactions between As-redox and P have been well studied, literature reports on the effect of P on As biomethylation are limited. Furthermore, to the best of our knowledge, N regulation on algal As transformation has not been investigated. Understanding such nutrient regulation is important for systematic research into As biogeochemical cycling, especially in freshwater with eutrophic status. In the present study, the growth differences of As-contaminated *M. aeruginosa* might be associated with varying response mechanisms of the algae to As stress under different nutrient treatments. Overall, the As(V) biotransformation pathway was largely in accordance with the model in previous studies (Cullen et al., 1994; Levy et al., 2005), in which As(V) is taken up by *M. aeruginosa* through a phosphate transporter, transformed *via* reduction and methylation in the cell, and subsequently excreted as As metabolites into the medium. Notably, our results showed different regulation of As transformation processes by N and P.

According to the statistical results of As(V) concentrations in the medium (Appendix A Table S3; $p > 0.05$) and TAs concentrations in the algae (Fig. 2a–c; 5.56–12.08, 6.03–10.69 and 6.73–11.47 μg/g for 4, 10 and 20 mg/L N, respectively), varying N levels did not induce significant regularity in As absorption by *M. aeruginosa*, but intracellular As(V) accumulation decreased with increasing N concentration from 4 mg/L (41%–76% of As uptake) to 10–20 mg/L (39%–68% and 41%–58%,

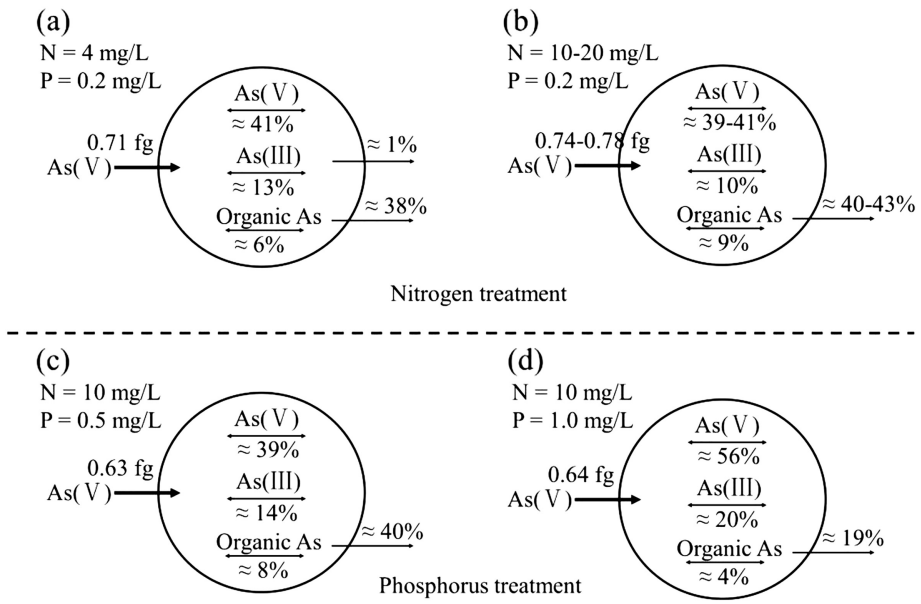

Fig. 5 – Arsenate fate in *M. aeruginosa* cells after 8-day incubation under different treatments. Organic As represents the sum of monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA).

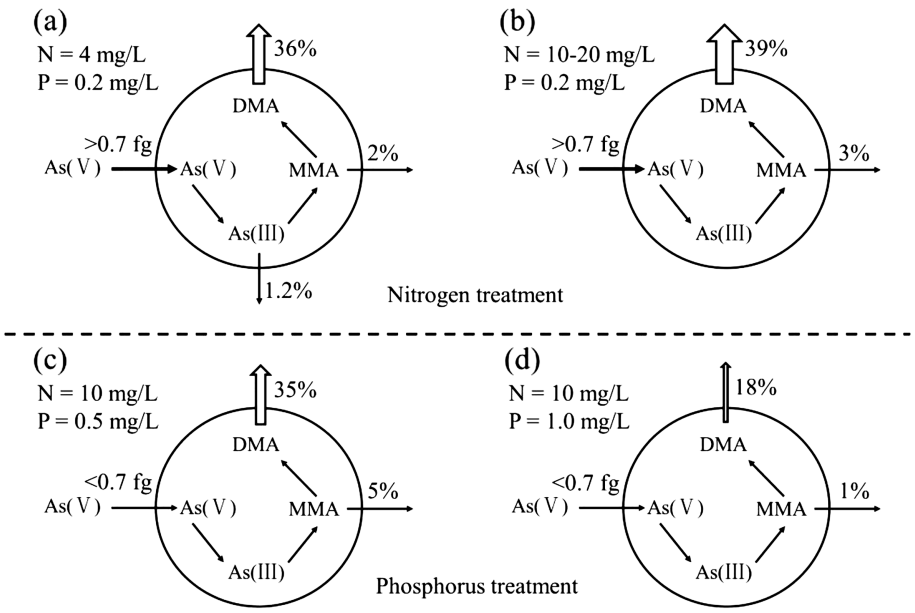

Fig. 6 – Arsenate metabolism pathways in *M. aeruginosa* after 8-day incubation under different treatments.

respectively). Maeda et al. (1993) also reported decreased As(V) accumulation in *Nostoc* sp. accompanying increased NO_3^- concentration in the medium, and it could be attributed to the stimulation of nitrogenase, which was only synthesized and activated in the absence of N. In the present study, however, since *M. aeruginosa* is a typical non-nitrogen-fixing cyanobacteria, the decreased intracellular As(V) content was probably due to the fast As(V) reduction stimulated by upgraded N levels. This was confirmed by the significantly higher As(III) concentration in *M. aeruginosa* cells at day 2 under 10–20 mg/L N treatment (5.68%–9.43% of As uptake), compared to that under 4 mg/L N treatment (4.06%). Similar positive correlation between N level and As(V) bio-reduction was reported by Wang et al. (2017). It was speculated that increased N concentration would stimulate the synthesis of antioxidants such as glutathione (GSH) (Downing et al., 2005), which serves as the source of reducing potential in As(V) bio-reduction (Rosen, 2002), but the underlying mechanisms require further investigation.

As shown in Fig. 2, the early production of intracellular As(III) showed a decreasing tendency from day 4 on, which was due to As(III) excretion and slower As methylation in *M. aeruginosa* (Hellweger et al., 2003). Since As(III) excretion cannot keep pace with As(V) reduction, As(III) accumulated in algae was then largely methylated to MMA and DMA and excreted into the medium at longer culture periods (Levy et al., 2005). In addition, it is noteworthy that N played an important role in As biomethylation. In our results, increasing N levels significantly enhanced the As methylation process under the condition of 0.2 mg/L P. On the one hand, upgraded N concentration obviously accelerated the cell growth of *M. aeruginosa* (Fig. 1), thus we speculated that a large number of *M. aeruginosa* cells in the culture with higher N level participated in the As biomethylation processes, resulting in significant increases in MMA and DMA concentrations in the medium (Fig. 3). In fact, increases in MMA and DMA were correlated with increases in cell density of *M. aeruginosa* in all 0.2 mg/L P treatments, with R of 0.670 and 0.960 respectively (Appendix A Fig. S2), demonstrating the positive effect of biomass concentrations on As methylation in *M. aeruginosa*. The result was in accordance with the reports by Hellweger et al. (2003) and Guo et al. (2011). On the other hand, as shown in Figs. 5 and 6, higher N treatments (10–20 mg/L) were correlated with higher intracellular organic As (about 9%) and organic As efflux (about 40%–43%) per cell of *M. aeruginosa*. The results suggested a positive relationship between increasing N concentration and improved As methylation ability in *M. aeruginosa* cells. Previous studies showed that As biomethylation in some cyanobacteria cells is due to the function of purified enzyme in the presence of S-adenosylmethionine (SAM) methyltransferases and GSH (Ye et al., 2012). Then a hypothesis could be considered that N sufficiency stimulated the synthesis of GSH (Liu et al., 2015a), which is important in As biomethylation processes, whereas the underlying mechanisms need to be further revealed. Besides, the excretion of DMA content was much higher than MMA content, which was due to the higher permeability coefficient for DMA compared to MMA, making the algae metabolize intracellular MMA to DMA and excrete it into the medium (Cullen et al., 1994). The higher average release rate of DMA supported the results (Fig. 4).

Different from the regulation of N, increasing P concentrations (0.5–1.0 mg/L) significantly inhibited As(V) uptake by *M. aeruginosa* (Fig. 6). This can be attributed to the physicochemical similarities between phosphate (PO_4^{3-}) and As(V) (AsO_4^{3-}), leading to their competition for phosphate transporters (Button et al., 1973). Therefore, higher rates of cell growth were also observed in 0.5–1.0 mg/L P treatments, because of relatively lower As stress. Similar findings have been reported for *C. reinhardtii*, *Scenedesmus obliquus*, and *Chlorella salina*, *etc.* (Karadjova et al., 2008; Wang et al., 2013a, 2014b). In addition, Wang et al. (2013a) proposed that the algal cells incubated at lower initial P level have to synthesize more phosphate transporters to compensate for the phosphate deficiency in the medium, resulting in higher As uptake in algal cells. Moreover, in

the present study, As(III) in the medium was detected on the first day under 0.2 mg/L P treatment (Fig. 3), in agreement with previous studies that intracellular As(V) reduction occurred rapidly in the early stage of incubation (Hasegawa et al., 2001; Hellweger et al., 2003). Subsequently, the As(III) concentration rapidly declined because of abiotic oxidation and possible surface oxidation in algae (Guo et al., 2011; Zhang et al., 2014). However, As(III) could not be detected in the medium under 0.5–1.0 mg/L P treatments, which suggested that higher levels of P impeded As(V) reduction and As(III) excretion by inhibiting As(V) uptake, similar to the finding for *Synechocystis* (Zhang et al., 2014). Additionally, Slaughter et al. (2012) also proposed the inhibition of As(V) reduction by P by competitively binding to the site of arsenate reductase.

Phosphorus also exhibited a negative impact on As biomethylation. In our results, higher P levels effectively inhibited As methylation in *M. aeruginosa* by impeding As(V) uptake and therefore contributing to lower percentages of MMA and DMA in the medium (Figs. 3 and 5), which is similar to the results by Wang et al. (2016). In addition, Hasegawa et al. (2001) also reported the relationship between increased DMA contents and decreased ratio of PO_4^{3-} to As(V), but Guo et al. (2011) considered that As metabolites in the medium were more affected by ambient P levels rather than the As(V) contents. Yan et al. (2014) also confirmed the effect of phosphate deficiency on the production of DMA in *M. aeruginosa*, similar to P regulation on As metabolism in rice species (Wang and Duan, 2009).

Overall, our results indicated that N sufficiency (10–20 mg/L) and P limitation (0.2 mg/L) significantly accelerated As(V) transformation in *M. aeruginosa* through reduction and methylation; in particular, P plays a key role in As(V) uptake and the species of As metabolites excreted from the cells. Interestingly, Baker and Wallschläger (2016) reported the As metabolism pathway in *C. vulgaris* cultures treated with 10 μg/L As(V) and <0.025–0.1 mg/L P, and the result exhibited increasing DMA excretion per cell (from 3%–6% to 20%) with upgraded P concentration (Appendix A Table S4). In comparison, with further increase of P concentration from 0.2 to 1.0 mg/L in the present study, the DMA excretion per cell markedly decreased from 36%–39% to 18%. The above results suggest a strong relationship between ambient P concentration and the regulation of As biomethylation by P.

4. Conclusions

This study demonstrated positive regulation by N (4–20 mg/L) and negative regulation by P (0.2–1.0 mg/L) on the As biotransformation by *M. aeruginosa*. Increasing N concentration not only accelerated the cell growth but also enhanced the methylation ability of the cells, leading to higher As methylation efficiency in *M. aeruginosa*. However, P showed a significant inhibiting effect on As uptake and subsequent biotransformation by *M. aeruginosa*. The findings of this study will provide a better understanding of As biotransformation by cyanobacteria, especially that related to algal bioremediation in As-contaminated water, because different levels of nutrients (including NO_3^- and PO_4^{3-}) may affect As biotransformation processes and the production of As metabolites.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2017.05.041.

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