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Cyanobacteria-Mediated Arsenic Redox Dynamics Is Regulated by Phosphate in Aquatic Environments

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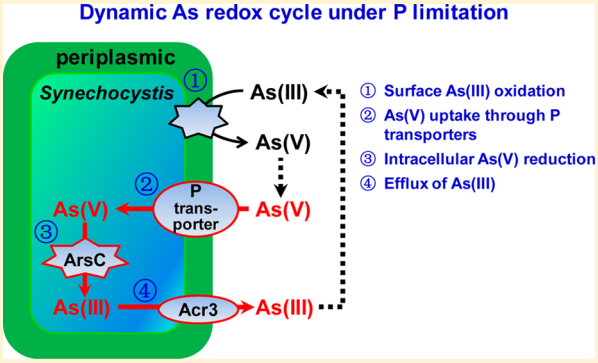
Supporting Information

ABSTRACT: Studies of cyanobacteria in environments where arsenic (As) and phosphate (P) both occur in significant concentrations have so far only focused on the effect of P on As(V) toxicity and bioaccumulation, with little attention to the influence of P on As redox transformations. Our study revealed that As(III) oxidation by *Synechocystis* appeared to be more effective with increased P levels. We demonstrated that the higher As(III) percentage in the medium under P-limited conditions was due to enhanced As(V) uptake and the subsequent efflux of intracellularly reduced As(III) which in turn contributed to higher As(III) concentrations in the medium. Arsenic redox changes by *Synechocystis* under P-limited conditions is a dynamic cyclic process that includes the following: surface As(III) oxidation (either in the periplasm or near the outer membrane), As(V) uptake, intracellular As(V) reduction, and As(III) efflux. These observations not only expand our understanding of how P influences microbial As redox metabolisms but also provide insights into the biogeochemical coupling between As and P in As contaminated eutrophic aquatic environments and artificial wetland-paddy fields.



INTRODUCTION

Arsenic (As) is a ubiquitous toxic substance broadly distributed in the environment.[1] The transport and transformation of As in environment are governed by geochemical and biological processes, generating an As biogeochemical cycle.[2] Microbially mediated biotransformations may play an important role in determining As biogeochemistry and toxicity in various natural ecosystems, especially As redox processes which influence the two most abundant inorganic As forms (arsenite As(III) and arsenate As(V)) in the environment.[3] Thus it is essential to understand microbial As redox metabolisms to make further advances in As bioremediation.

Microalgae are desirable organisms for As remediation in contaminated water because of their high surface area to volume ratios.[4] Studies have shown that microalgae can tolerate high levels of As and biotransform it in several ways to detoxify it.[5−9] Considering the complex living environments of microalgae, many factors could affect As biotransformations by microalgae. Phosphate (P) is one of the most significant determinants of microalgal growth. Due to its structural similarity with As(V),[10] the influence of P on microbial As metabolisms has been extensively studied and primarily focused on As(V) accumulation and toxicity.[11,12]

Besides affecting As(V) uptake, P can further influence the subsequent As detoxification process, i.e. As(V) reduction.[13,14] In the bacteria *Agrobacterium tumefaciens* str. 5B, it has been reported that As(V) reduction was completely suppressed at elevated levels of P (500 and 1000 μM), but the expression of the As(V) reductase gene (*arsC*) was not inhibited by high P concentrations.[15] As(III) oxidation and the Phosphorus (Pi) concentration were reported to be tightly and intimately coregulated in the soil bacterium *Agrobacterium tumefaciens*.[16] However, when analyzing As(III) oxidation under different P levels, Slaughter et al.[15] suggested that the presence of P had little apparent effect on As(III) oxidation in the same oxidizing strain of *Agrobacterium tumefaciens*. Little has been studied regarding the influence of P on As redox processes in

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cyanobacteria or other microalgae. Even less is known about how P mediates the dynamics of As redox processes in cyanobacteria.

Cyanobacteria are widely distributed in the environment,[17,18] and they are dominant in algal blooms, which are primarily caused by high P levels,[19] especially in aquatic environments of higher trophic levels.[20,21] Considering cyanobacterial blooms and As pollution coexist in many freshwater lakes in China,[12] it is important to understand the effect of P on As transformations in cyanobacteria and therefore facilitate the application of cyanobacteria in As remediation. In this study, we chose the typical freshwater cyanobacteria *Synechocystis* sp. PCC6803 to study the influence of P concentrations on As redox processes. Furthermore, the genes encoding As(V) reductase (*arsC* slr0946) and As(III) efflux pump protein (*acr3* slr0944) have been identified in *Synechocystis*,[22] and As resistance in *Synechocystis* has been reported to be mediated by an operon of the three genes *acr3* (slr 0944), *arsH* (slr0945), and *arsC* (slr0946), which is regulated by ArsR.[22,23] Considering this, we used quantitative real-time polymerase chain reaction (qPCR) to analyze the expression of *arsC* under + P and − P conditions and provide further insights on the effect of different P concentrations on As redox processes in *Synechocystis*.

MATERIALS AND METHODS

Cultivation and Growth Conditions. *Synechocystis* was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China and grown in 100 mL of BG-11 medium (230 μM K_2HPO_4)[24] in 250 mL conical flasks. Cultures were maintained in a controlled-environment growth chamber under the following conditions: 16 h light period day^{-1} with a light intensity of approximately 280 mmol m^{-2} s^{-1}, 25 °C/20 °C of day/night temperatures, and 60% relative humidity. The inoculum used in the experiments came from an exponential phase culture.

Measurement of As(III) Oxidation under Different P Concentrations. Cells of *Synechocystis* were precultured in BG-11 medium for 7 days, then collected by centrifugation (6000 rpm), and subsequently rinsed with BG-11 medium without phosphorus addition for three times to remove P on the cyanobacterial surface. The collected cyanobacterial cells were then transferred to 100 mL medium without P for 3 days in order to consume all remaining phosphorus *in vivo*. After preculture, cells of *Synechocystis* were incubated at an initial optical density (OD_{730}) of 0.260 in a medium consisting of 10 μM As(III) ($NaAsO_2$) with different added concentrations of P (0, 10, 50, 75, 100, 150, 230, 690 μM). P was supplied as K_2HPO_4. To ensure the concentration of maintained K^+ in BG-11 medium (460 μM), KCl was added to each sample to ensure equal concentration of K^+. For instance, 230 μM K_2HPO_4 was replaced with 460 μM KCl to acquire 0 μM P BG-11 medium. Three replicates were measured for each treatment. After exposure for 7 days, aliquots of 2 mL medium were taken and filtered through 0.45 μm nylon filter and kept in the freezer at −80 °C for analysis of As speciation. The percentage of oxidized As(V) to total As in medium was evaluated.

Short Time-Course of As Redox by *Synechocystis* under Different P and DNP Treatments. DNP (uncoupling agent 2,4-dinitrophenol) is a respiration inhibitor and an uncoupler of oxidative phosphorylation. It can cause dissipation of the proton motive force across the membranes and thus inhibits As uptake by microorganism.[25] In order to locate As(III) oxidation by *Synechocystis*, the effect of DNP on As redox transformations by *Synechocystis* under different P conditions was investigated. *Synechocystis* was exposed to 2 μM As(III) ($NaAsO_2$) or As(V) (Na_3AsO_4) for 48 h in medium with four different treatments, including − P − DNP (medium without P and DNP addition), − P + DNP (medium with DNP addition, and no P addition), + P − DNP (medium with P addition, and no DNP addition), and + P + DNP (medium with P and DNP addition). DNP was supplied at 500 μM, and P was supplied at 230 μM (the normal P concentration in BG-11 medium). Each treatment was replicated 3-fold. Aliquots of 2 mL medium were taken at 0, 8, 16, 24, 48 h, filtered through a 0.45 μm nylon filter and kept in the freezer at −80 °C for later analysis. Control treatment (without cyanobacterial cells) and dead cells treatment (sterile medium after inoculation with *Synechocystis*) were carried out in the same way as described above. After exposure for up to 48 h, cyanobacterial cells were collected and processed as described above. As speciation both *in vivo* and in the medium was determined. The percentages of As(III) and As(V) to total As in the medium were calculated respectively to evaluate the efficiency of As(III) oxidation and As(V) reduction.

Determination of As Speciation in Medium and in Cells after Exposure to As(III) under Different P Concentrations. *Synechocystis* (10 mL at the exponential growth stage) was inoculated in 90 mL of P modified BG-11 medium adjusted as follows: 5 μM of PO_4^{3-} as the P-limited condition (replaced with equal concentration of K^+ using KCl), 230 μM of PO_4^{3-} as the P-rich condition. Arsenic was supplied as $NaAsO_2$ at 10 μM. Three replicates were used in a completely randomized experimental design. After exposure to As(III) for 0, 2, 4, 6, 8, 10 days, aliquots of 2 mL of medium were taken and cyanobacteria were collected by centrifugation (6000 rpm) and subsequent rinsing with deionized water and ice-cold P buffer (1 mM K_2HPO_4, 5 mM MES and 0.5 mM $Ca(NO_3)_2$) for 10 min to remove apoplastic As. Cyanobacterial cells were freeze-dried and kept in 50 mL polypropylene tubes with 10 mL of 1% HNO_3 overnight. The samples were extracted using a microwave (CEM Microwave Technology Ltd., Matthews, NC, USA). The working program was as follows: 55 °C for 10 min, 75 °C for 10 min, and 95 °C for 30 min, with 5 min ramp time between each stage.[26] Supernatants from medium and cellular extractions were filtered through 0.45 μm nylon filter and kept in the freezer at −80 °C for As speciation analysis. Growth of *Synechocystis* at 0, 2, 4, 6, 8, 10 days was measured as optical density at 730 nm (OD_{730}).

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction (qPCR). RNA was extracted from *Synechocystis* cultivated under four treatments (+ P + As(III), + P − As(III), − P + As(III), − P − As(III)) after 7 days. Arsenite was supplied at 10 μM, and P was supplied at 230 μM. The cell pellet was collected and frozen in liquid nitrogen. Total RNA was isolated from *Synechocystis* using TRIzol reagent (Invitrogen), following the manufacturer's recommendations. DNaseI (Promega, USA) was used to remove contaminating genomic DNA. The extracted RNA was reverse transcribed using a PrimeScript RT-PCR Kit (Promega, USA) and random primers. Primers (*arsC*-F, *arsC*-R, *rnpB*-F, *rnpB*-R) (Table S1) were selected to amplify a 150 bp region inside the predicted region of transcriptional overlap of *arsC* and *rnpB*. qPCR with SYBR Green I was performed on iQTM5 Thermocycler (Bio-Rad, USA) using SYBR Premix Ex TaqTM (Takara Bio Inc., Japan). Each qPCR was performed

in triplicate with *rnpB* as the internal standard. The PCR amplification profile was 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, 72 °C for 30 s, 80 °C for 10 s. The relative changes in gene expression were calculated using the $2^{-[\Delta CP\ sample - \Delta CP\ control]}$ formula.[27]

Arsenic Speciation Analysis. Arsenic speciation was determined by HPLC-ICP-MS (7500a; Agilent Technologies) as described.[26] Chromatographic columns were obtained from Hamilton and consisted of a precolumn (11.2 mm, 12−20 μm) and a PRP-X100 10-μm anion-exchange column (250 × 4.1 mm). The mobile phase consisted of 10 mM diammonium hydrogenphosphate ($(NH_4)_2HPO_4$) and 10 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using nitric acid. The mobile phase was pumped through the column isocratically at 1 mL min^{-1}. Arsenic species in the samples were identified by comparing their retention time with those of the standards, including arsenite (As(III)), arsenate (As(V)), and quantified by external calibration curves with peak areas.

RESULTS

As(III) Is Oxidized by *Synechocystis*. Fresh cells (*Synechocystis*), dead cells, and control treatment (without cyanobacterial cells) were prepared to investigate As(III) oxidation (Figure S1). After exposure to 2 μM As(III) for 48 h, 45.0% of As(III) was oxidized to As(V) by *Synechocystis*, which was significantly ($P < 0.01$) higher than that of dead cells (4.7%) and control (3.6%) treatments, indicating that As(III) oxidation was attributed to fresh cells of *Synechocystis* but not dead cells or chemical oxidation.

The Percentage of As(V) in the Medium Was Higher under Elevated P Concentrations. After exposure of *Synechocystis* to 10 μM As(III) under different P concentrations for 7 days, the percentage of As(V) to total As in the medium was determined. The percentage of As(V) increased with increasing P concentrations (0−100 μM) (Figure 1). When the supplied P concentration was higher than 100 μM (including 100 μM), almost 100% of total arsenic was As(V) after incubation for 7 days.

Addition of DNP Improved Visible As(III) Oxidation but Not As(V) Reduction in *Synechocystis* under P-Limited Conditions. Under P-limited conditions, the percentage of oxidized As(V) increased to 23.2% within 16 h and then decreased to 8.7% at 48 h in medium with no DNP addition (− P − DNP) (Figure 2). This was possibly due to

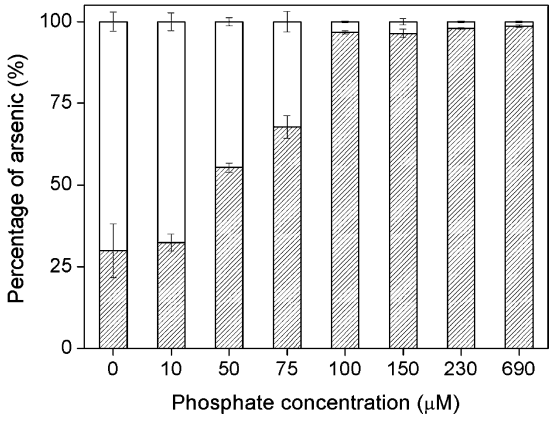

Figure 1. The percentage of As species (hatched bars, As(V); open bars, As(III)) to total As in medium after exposure to 10 μM As(III) for 7 days under 0, 10, 50, 75, 100, 150, 230, 690 μM P. All data are means ± standard deviation ($n = 3$).

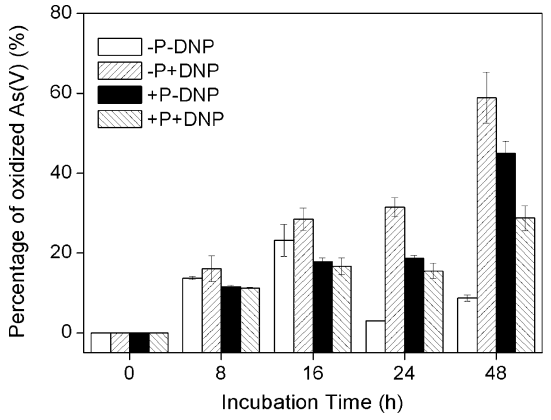

Figure 2. The percentage of oxidized As(V) to total As in medium incubated with *Synechocystis* sp. PCC6803 after exposure to 2 μM As(III) for 0, 8, 16, 24, 48 h under four treatments (− P − DNP, − P + DNP, + P − DNP, and + P + DNP) during a 48 h cultivation. DNP was supplied at 500 μM, and P was supplied at 230 μM (the normal P concentration in BG-11 medium). All data are means ± standard deviation ($n = 3$).

Synechocystis first oxidized the added As(III) to As(V). Then the uptake of oxidized As(V) by *Synechocystis* was enhanced by P-limited condition, followed by intracellular As(V) reduction and As(III) efflux (supported by the results in Figures S2, S3, and S4), which contributed to the lower As(V) percentage in the medium, i.e. the decrease of oxidized As(V) at 48 h. However, under − P + DNP treatment, 58.9% of the 2 μM As(III) was oxidized to As(V) within 48 h, higher than the other treatments (− P − DNP (8.7%), + P − DNP (45.0%), and + P + DNP (28.8%)). Under P-rich conditions, despite DNP addition, As(V) percentage showed no significant difference between + P − DNP and + P + DNP treatments within 24 h, indicating that DNP did not shift the As(III)/As(V) ratio in medium. The lower percentage of As(V) under + P + DNP treatment detected on 48 h might be attributed to poor growth of *Synechocystis* affected by DNP.

Under P-limited conditions with no DNP addition, the percentage of reduced As(III) increased with incubation time and reached 97.7% at 24 h (Figure S2). However, when DNP was added to the P-limited medium (− P + DNP), As(V) reduction could not be detected during the incubation time. Under P-rich conditions, despite DNP addition, As(V) was always the dominant As species under either + P − DNP or + P + DNP treatments.

DNP and P Inhibited Intracellular As Accumulation. After exposure to As(III), total As in cells was 4.6, 0.7, 0.6, and 0.1 mM DW, respectively, under − P − DNP, − P + DNP, + P − DNP, and + P + DNP treatments (Figure S3). When supplied with As(V), total As in cells was 7.9, 1.9, 0.5, and 0.1 mM DW, respectively (Figure S4). Regardless of the species of As exposure, the intracellular As concentrations under + DNP treatments were lower than those under − DNP treatments (+ P + DNP < + P − DNP, − P + DNP < − P − DNP), indicating DNP inhibited As uptake by *Synechocystis*. Arsenic accumulation in cells was lower under P-rich conditions than under P-limited conditions (+ P + DNP < − P + DNP, + P − DNP < − P − DNP), suggesting that P suppressed As uptake, especially

As(V) uptake. Among all the different treatments, As(V) was always the dominant As species in cells.

Time-Course of Apparent As(III) Oxidation in Medium Differed under P-Rich and -Limited Conditions. *Synechocystis* was incubated in a medium with 10 μM As(III) under either 230 μM (P-rich) or 5 μM (P-limited) P conditions, and the concentration of As(III) was monitored over a 10-day period (Figure 3). The percentage of As(V) in the medium

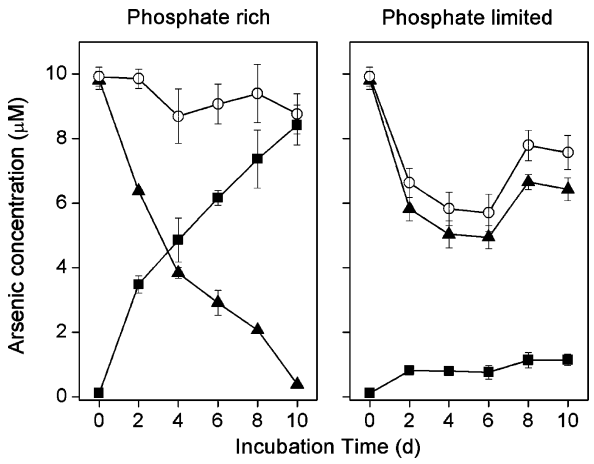

Figure 3. Arsenic species (▲, As(III); ■, As(V); ○, sum of different As species) in medium with *Synechocystis* sp. PCC6803 after exposure to 10 μM As(III) under P-limited (5 μM P) and P-rich (230 μM P) treatments during a 10 d cultivation. Arsenic species in medium were analyzed on 0, 2, 4, 6, 8, 10 days. All data are means ± standard deviation (n = 3).

under P-rich conditions increased more rapidly than that under P-limited conditions. Under P-rich conditions, As(III) concentration decreased with a concomitant increase of As(V) concentration in the medium, from 0.1 to 8.4 μM, and by day 6, 94% of the 10 μM As(III) added was present as As(V). Under P-limited conditions, As(III) remained to be the predominant As species (85−88% of the total As) during the incubation time, and the As(V) concentration was stable at 0.8−1.1 μM at day 2−10 (12−15% of the total As). The As(III) concentration in the medium decreased from 10 to 4.9 μM after the first 6 days. After 8 days incubation, the As(III) concentration increased to 6.7 μM. Total As concentration under P-limited conditions decreased from 10 μM to 5.7 μM within 6 days, which was almost 4 times higher than the decrease (1.2 μM) under P-rich conditions, indicating more As uptake by *Synechocystis* under P-limited conditions.

Time-Course of As Species Accumulated in *Synechocystis* Was Significantly Different under P-Rich and -Limited Conditions. P limitation had a significant effect on total As concentration as well as on As speciation in cyanobacterial cells (Figure 4). Under P-limited conditions, total As concentration in cells increased rapidly within the first 6 days, reaching up to 7.9 mM DW. This concentration was about 10 times higher than that under P-rich conditions (0.7−0.9 mM DW). During incubation, As speciation was dominated by As(V) (82−90% of the total As) in cyanobacterial cells under P-limited conditions. In contrast, under P-rich conditions, As(V) represented 51−65% of the total As in cells. Both As(V) and As(III) concentrations in cells showed a decrease after incubation for 6 days under P-limited conditions, and the total As concentration dropped to 5.8 mM DW at day

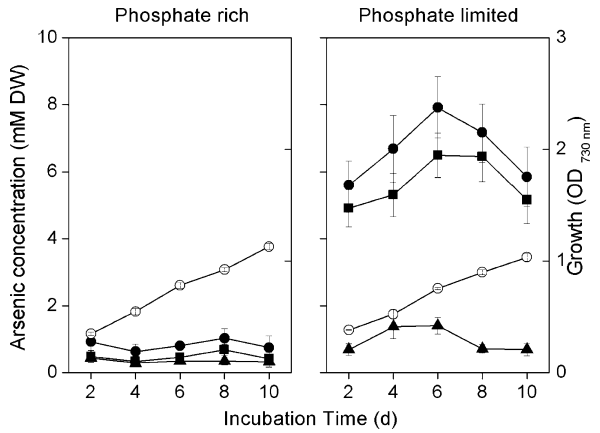

Figure 4. Arsenic species (▲, As(III); ■, As(V); ●, sum of different As species) in cyanobacterial cells and the growth curve of *Synechocystis* sp. PCC6803 (○, $OD_{730\ nm}$) after exposure to 10 μM As(III) under P-limited (5 μM P) and P-rich (230 μM P) treatments during a 10 d cultivation. Arsenic species in *Synechocystis* were analyzed on 2, 4, 6, 8, 10 days. All data are means ± standard deviation (n = 3).

10, indicating that *Synechocystis* might efflux part of the As(V) and As(III) from cells to the medium, as a possible As detoxification mechanism in cyanobacteria.[5] During the incubation time (10 d), $OD_{730\ nm}$ of *Synechocystis* under 230 μM and 5 μM P conditions showed no significant difference (Figure 4).

Transcription Analysis of *arsC* under Different P and As(III) Levels. The expression of gene involved in As(V) reduction (*arsC*) under different P and As(III) levels (+ P + As(III), + P − As(III), − P + As(III), − P − As(III)) was analyzed using qRT-PCR (Figure 5). With As(III) addition, the

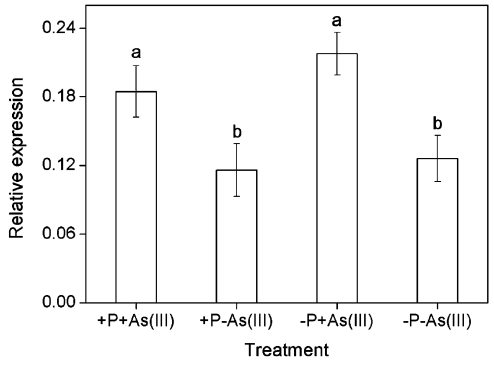

Figure 5. qPCR analysis to investigate *arsC* transcriptions. Total RNA was isolated from *Synechocystis* sp. PCC6803 under four treatments (+ P + As(III), + P − As(III), − P + As(III), − P − As(III)) after incubation for 7 days. Arsenite was supplied at 10 μM, and P was supplied at 230 μM. All data are means ± standard deviation (n = 3). The transcription level of *arsC* under + As(III) treatments were significantly (P < 0.01) different from treatments of − As(III).

two P levels did not significantly influence *arsC* expression in *Synechocystis*. However, regardless of P levels, the addition of As(III) significantly (P < 0.01) enhanced *arsC* transcription levels in *Synechocystis*.

■ DISCUSSION

Our results indicated that As(III) oxidation was mediated by fresh cyanobacterial cells, because neither dead cells (Figure S1) nor cyanobacterial excretion had any effect on it,[5] and

As(III) oxidation by *Synechocystis* was much more rapid than chemical oxidation (Figure S1). As an uncoupler of oxidative phosphorylation, DNP can cause dissipation of the proton motive force across the membranes and thus inhibits As uptake by *Synechocystis* but should not change As redox processes. Based on the fact that DNP had no significant effect on 24 h As(III) oxidation under + P − DNP and + P + DNP treatments (Figure 2), we therefore suggest that As(III) oxidation in *Synechocystis* is likely a surface oxidation process (either in the periplasm or near the outer membrane) rather than intracellular As(III) oxidation following As(III) uptake. As(III) oxidation in many microorganisms, such as *Agrobacterium tumefaciens* 5A,[28] *Herminiimonas arsenicoxydans* ULPAs1,[29] *Rhizobium* sp. NT-26,[30] and *Thiomonas* sp. 3As,[31] has been reported to be mediated by the respiratory As(III) oxidase which is considered to be a periplasmic protein (found outside the inner cell membrane but associated with it).[32,33] However, a related As(III) oxidase in *Synechocystis* has yet to be identified and perhaps does not exist. Of course, other, unrelated enzymes might be responsible for As(III) oxidation in *Synechocystis* and perhaps other cyanobacteria.

Phosphate as an important environmental factor could influence As(V) uptake and reduction in microorganisms.[15,16,34,35] Our study indicated that the presence of P could inhibit intracellular As(V) reduction (Figure S2) by decreasing As(V) uptake in *Synechocystis* (Figure S4). The impeding of As(V) uptake by P might via P transport systems or by competitively binding to the active site of ArsC.[15,35] Our results also revealed that a higher P concentration resulted in a higher As(V) percentage in the medium as a result of As(III) oxidation by *Synechocystis* (Figure 1). The difference in As(III) oxidation under P-limited and P-rich conditions more likely resulted from a P effect rather than the difference in growth of *Synechocystis*, since $OD_{730\ nm}$ of *Synechocystis* in the two media showed no difference during the incubation period (Figure 4). We further suggest that the lower As(V) percentage in the medium, i.e. lower As(III) oxidation, under P-limited conditions is not due to the direct inhibition of low P on As(III) oxidation. The possible pathway of this influence is as follows: under P-limited conditions, uptake of oxidized As(V) was enhanced; therefore, intracellular As(V) reduction was increased and then followed by As(III) efflux. This is supported by the results from the − P + DNP treatment, in which DNP inhibited As uptake and reduced intracellular As(V) reduction in *Synechocystis* (Figures S2, S3, and S4), thus leading to a higher percentage of As(V), i.e. higher As(III) oxidation in the medium under P-limited conditions (Figure 2). Under P-rich conditions, the presence of P inhibited intracellular As(V) reduction and subsequent As(III) efflux by impeding the uptake of oxidized As(V) from the medium and therefore contributing to the higher As(V) percentage in the medium, i.e. higher net As(III) oxidation (Figures 2 and 3). Considering *Synechocystis* has been recognized as a typical freshwater cyanobacterium, we believe that P will influence As redox processes in other cyanobacteria as well.

The higher percentage of As(III) in the medium under P-limited conditions was likely due to improved As(V) uptake, and subsequent efflux of intracellular reduced As(III) in a dynamic cycle of an As redox process under P-limited conditions. We propose the following model to depict the predominance of As(III) under P-limited conditions. First, As(III) was oxidized to As(V) by surface oxidation as discussed above. Second, more As(V) was taken up into cells under P-limited condition (Figure 4) through the upregulated P transporter system,[12] resulting in a decreased As(V) concentration in the medium (Figure 3). The intracellular As(V) was then reduced to As(III) and subsequently effluxed out of the cells into the medium for detoxification[13,14] and thus resulted in the higher As(III) concentration in the medium (Figure 3). Furthermore, As(III) effluxed to the medium may once more be oxidized by *Synechocystis* and generate a dynamic cycle of As redox processes. Intracellular As(V) reduction and As(III) efflux were improved by As(III) addition, as indicated by a significantly higher expression of *arsC* under + As(III) treatment (Figure 5), and led to an As redox cycle in *Synechocystis*. The expression of *acr3* (encoding the As(III) efflux pump) could be similar to *arsC*, since it is in the same operon in *Synechocystis*.[22,23] Phosphate levels did not influence *arsC* expression (Figure 5), which had also been demonstrated in *Agrobacterium tumefaciens* str. 5B and *Shewanella* sp. Strain ANA − 3.[15,36]

Our results also revealed that As uptake in *Synechocystis* was an active process, because DNP significantly inhibited both As(III) and As(V) accumulation in cyanobacterial cells (Figures S3 and S4). As(III) uptake has been generally accepted as an energy-dependent process, mainly via aquaglyceroporins (transport of water, glycerol, and other small uncharged molecules) in bacteria.[37] As(V) uptake can be catalyzed by two P transporters in *E. coli*, the P-specific transport (Pst) system and P-inorganic transport (Pit) system.[38] In the cyanobacteria *Microcystis aeruginosa*, Guo et al. have proposed that As(V) absorption was through the Pit system under 0, 1, and 10 μM P, while the Pst system was the major uptake pathway under 175 μM P.[12] For *Synechocystis*, higher As(V) accumulation in cells under P-limited conditions (Figure 4) might be attributed to As(V) absorption through the Pit system which has lower affinity to P.

In conclusion, our results demonstrated that in the presence of cyanobacterium-*Synechocystis*, arsenic redox dynamics was influenced by P levels. As(III) oxidation by *Synechocystis* appeared to be more effective with increased P levels, indicating that P concentrations should be taken into consideration when predicting the effect of biological As(III) oxidation, such as under algal bloom conditions. Since As(V) is less toxic to humans and much easier to remove by conventional treatments, such as coagulation, coprecipitation, and adsorption process,[39−41] there is a possibility of increasing As(V) in the environment by cyanobacterial oxidation through regulating P concentrations and thus facilitate As removal from solution. Moreover, considering *Synechocystis* is the model cyanobacterium, and widely spread in some P and arsenic coexisting environments, such as a eutrophic aquatic environment as well as artificial wetland-paddy fields,[17,18] we reasonably speculate the biogeochemical coupling of arsenic and phosphorus exists in these aquatic and wetland environments.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Sequences of primers used for real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR); the results of As(III) oxidation by *Synechocystis*, dead cells, and control treatment; the results of As(V) reduction by *Synechocystis* under − P − DNP, − P + DNP, + P − DNP, and + P + DNP treatments; As species in cyanobacterial cells under − P − DNP, − P + DNP, + P − DNP, and + P + DNP treatments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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