

## Article

# Effects of Phosphate and Arsenate on As Metabolism in *Microcystis aeruginosa* at Different Growth Phases

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**Abstract:** Arsenic (As) metabolism in freshwater algae at different growth phases has rarely been documented. To address this gap, this study was conducted to assess the intra- and extracellular As metabolism, along with speciation changes, in *Microcystis aeruginosa* across three growth phases. The treatment involved varying concentrations of As (0, 0.4, 0.6, 0.8 and 1 mg/L, in the form of arsenate, iAs<sup>V</sup>) under three phosphorus levels (0.02 mg/L as low, 0.1 mg/L as medium, and 0.5 mg/L as high P in the form of phosphate). The findings revealed that extracellular iAs<sup>V</sup> remained the dominant As species during the lag and exponential growth phases of *M. aeruginosa* in the growth media, while intracellular trivalent As (iAs<sup>III</sup>) emerged as the pronounced species during the exponential growth phase, but also exhibited a significant negative correlation with the P levels. Moreover, elevated P levels had promoted the formation of intra- and extracellular dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) in the exponential growth phase. During the stationary growth phase, intracellular iAs<sup>V</sup> was found to decrease with the increasing P levels. During the whole growth phases, P had consistently reduced algal As absorption levels. The significant promotion of algal As absorption in response to iAs<sup>V</sup> was observed only during the lag growth phase. The As bioaccumulation exhibited a correlational relationship with the algal reproduction. Both low and high P levels (0.02 and 0.5 mg/L) decreased the accumulation of As in algae cells during the exponential and stationary growth phases. The transformation and release rate of As were concomitantly influenced by P, and exhibited the same trends within the growth phase. These trends differed between the exponential and stationary growth phases, with an inhibitory effect being present during the former, while a promotional effect was observed during the latter. This study provides insight into potential As hazards in freshwater lakes with algae bloom.

**Keywords:** arsenic; *Microcystis aeruginosa*; phosphorus; metabolism; growth phase



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## 1. Introduction

Arsenic (As) is a pervasive substance in the environment, and is toxic and carcinogenic. The widespread contamination of terrestrial and aquatic environments with As has received increasing attention over time [1]. In natural waters, As can be found in both inorganic forms (e.g., arsenite (iAs<sup>III</sup>) and arsenate (iAs<sup>V</sup>)) and organic forms (e.g., dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA)) [2]. iAs<sup>V</sup> dominates in oxidizing environments, and iAs<sup>III</sup> dominates in reducing environments [3]. Arsenic toxicity is highly dependent on its chemical form. When compared to inorganic forms, organic forms are relatively less toxic. The International Agency for Research on Cancer (IARC 2012) has categorized inorganic As (iAs) as a Group I carcinogen for causing skin cancer, kidney cancer, and bladder cancer. Generally, water contaminated with As contains more than 10 µg/L As (WHO guideline value) [4], and, in some regions, these levels can

reach as high as 1480 µg/L. Therefore, proper remediation techniques must be developed for the removal of iAs from water in order to reduce the human health risks associated with As.

The algal bioremediation of polluted water has gained more attention in recent years, largely as this it may present a promising and environmentally friendly method [5]. *Lessonia nigrescens*, *Chlorella* sp., *Scenedesmus* sp., and *M. aeruginosa* have been reported to have high As accumulation rates, with potential to be useful in water treatment processes [6,7]. Specifically, *M. aeruginosa* often dominates in freshwater ecosystems and causes algal blooms, while also showing high tolerance levels to iAs<sup>V</sup> [8]. Ongoing research has highlighted the potential of this algae to remove and detoxify iAs<sup>V</sup> via accumulating and transforming the pollutant to a less toxic methylated As species (i.e., MMA<sup>V</sup> and DMA<sup>V</sup>) [9]. Accordingly, *M. aeruginosa* can play a critical role in the absorption of As in aquatic ecosystems [6].

Phosphate (PO<sub>4</sub><sup>3-</sup>), a chemical analog of arsenate, has been documented to significantly influence the absorption and biotransformation of iAs<sup>V</sup> in algae [10]. Once inside the cell, iAs<sup>V</sup> has the capacity to act as a substitute for PO<sub>4</sub><sup>3-</sup> in various biochemical reactions, some of which lead to the toxic effects of As. For instance, iAs<sup>V</sup> substituted for PO<sub>4</sub><sup>3-</sup> interferes with ATP and nucleic acid synthesis. Phosphate, a crucial chemical known to limit growth in numerous eutrophic freshwater environments [11], has the potential to promote the proliferation of undesirable organisms, leading to ecological dominance. Simultaneously, phosphate serves as a phosphorus source for certain algae and cyanobacteria, supporting primary production [12].

Over the past few decades, there has been extensive research and tentative discussions regarding the effects and mechanisms of PO<sub>4</sub><sup>3-</sup> on iAs<sup>V</sup> metabolism in algae [13–15]. The chemical similarity between iAs<sup>V</sup> and PO<sub>4</sub><sup>3-</sup> has led to varying bioaccumulation and biotransformation changes in algae. These factors, in turn, affect the biogeochemical cycling of iAs<sup>V</sup> and algal potential for As absorption [16]. Despite the significant implications, only a limited number of studies on the effects of PO<sub>4</sub><sup>3-</sup> on iAs<sup>V</sup> metabolism in algae have been published to date. The presence of PO<sub>4</sub><sup>3-</sup> in the aquatic environment not only influences the iAs<sup>V</sup> metabolism of algae, but also impacts algal growth [17,18]. Therefore, it is imperative to investigate the iAs<sup>V</sup> metabolism in algae under different phosphate regimes in order to further advance iAs<sup>V</sup> absorption.

In this study, we propose a hypothesis that the iAs<sup>V</sup> metabolism of *M. aeruginosa* during different growth phases is influenced by extracellular concentrations of phosphate and arsenate. To test this hypothesis and gain a deeper understanding of the ecological risks associated with algal blooms and As metabolism, we conducted experiments focusing on changes in intracellular and extracellular As speciation, as well as absorption, bioaccumulation, transformation, and the release of As by algae at three growth phases (lag, exponential, and stationary growth phase) under varying phosphate and arsenate conditions. The primary objectives of these experiments were (1) to evaluate the impact of phosphate on the algal iAs<sup>V</sup> metabolism; (2) to evaluate the impact of arsenate on the algal iAs<sup>V</sup> metabolism; and (3) to provide additional insights into the biogeochemical cycle of As in freshwater systems.

## 2. Materials and Methods

### 2.1. Algae Culture and Pretreatment

A strain of *M. aeruginosa* (FACHB-1322) from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences, was selected for this study. This strain is a widely prevalent algae species in freshwater lakes in China. Before initiating the experiments, algal cells of *M. aeruginosa* (FACHB-1322) with the optical cell density (OD<sub>680</sub>) of 0.10 were centrifuged for 15 min at 5000 r·min<sup>-1</sup>. Subsequently, the algal cells were washed twice with the same volume of sterile ultrapure water in order to minimize any residual As and P attached to cells. The washed *M. aeruginosa* cells were then transferred to a modified sterilized M11 growth medium [MgSO<sub>4</sub>·7H<sub>2</sub>O (11.25 mg), K<sub>2</sub>HPO<sub>4</sub> (1.5 mg),

NaNO<sub>3</sub> (15 mg), CaCl<sub>2</sub>·2H<sub>2</sub>O (6 mg), Na<sub>2</sub>CO<sub>3</sub> (3 mg), Na<sub>2</sub>·EDTA·2H<sub>2</sub>O (0.15 mg), all reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China] at 25 °C under a photon flux density of 40 μmol/m<sup>2</sup>/s, provided by cool white, fluorescent irradiation, with a 12 h:12 h light/dark interval orbital incubation (125 rpm). To ensure adequate gas exchange, the test flasks were manually rotated and shaken three times during each light cycle. Prior to use, all stock vessels and culture flasks were meticulously cleaned by being soaked in 10% HCl for at least 24 h, followed by rinsing with Milli-Q water, oven drying, autoclaving, and handling under sterile conditions.

## 2.2. Experimental Design

The algal cells of *M. aeruginosa* (FACHB-1322) under phosphorus-starved conditions were centrifuged and washed twice with sterile deionized water. Subsequently, they were divided equally into 36 portions (3 P treatments, each with 12 parallels, as shown in Table 1 [19]), and incubated in modified M11 media with KH<sub>2</sub>PO<sub>4</sub> (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) as the phosphorus source for 96 h. The media contained 0.02, 0.1, and 0.5 mg/L of P in the form of PO<sub>4</sub><sup>3-</sup> to simulate As metabolism in freshwater algae, subject to the influence of varying P levels corresponding to different eutrophication statuses (low, medium, and high).

**Table 1.** Experimental design of the interaction between *M. aeruginosa* and iAs<sup>V</sup>.

| Serial Number | iAs <sup>V</sup> Levels (mg/L) | P Levels (mg/L) |
|---------------|--------------------------------|-----------------|
| 1             |                                | 0.02            |
| 2             | 0.4                            | 0.1             |
| 3             |                                | 0.5             |
| 4             |                                | 0.02            |
| 5             | 0.6                            | 0.1             |
| 6             |                                | 0.5             |
| 7             |                                | 0.02            |
| 8             | 0.8                            | 0.1             |
| 9             |                                | 0.5             |
| 10            |                                | 0.02            |
| 11            | 1                              | 0.1             |
| 12            |                                | 0.5             |

The As<sup>V</sup> solution was prepared by dissolving Na<sub>3</sub>AsO<sub>4</sub>·12H<sub>2</sub>O (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) in deionized water. Subsequently, *M. aeruginosa* was exposed to 0.4, 0.6, 0.8, and 1 mg/L iAs<sup>V</sup> at specific As addition time points during each growth period [19] (lag, exponential, and stationary phases, as outlined in Table 2 [19]). The exposure took place in 250 mL Erlenmeyer flasks, containing 100 mL M11 medium with three different P levels, and each treatment was replicated three times (Table 1). After a 2-day exposure to As, 30 mL algal cells from each replicate were harvested via centrifugation (7000× g). Subsequently, the cells were cleaned three times with ice-cold 0.1 mol/L phosphate buffer (pH 7.0, 3.5% salinity to maintain the osmotic balance and cell integrity) for 2 min in order to eliminate any As bound onto the cell surface [15]. Duplicate samples were then freeze-dried, and stored in a desiccator before As speciation analyses.

**Table 2.** iAs<sup>V</sup> addition time under different P levels.

| P Levels (mg/L) | iAs <sup>V</sup> Add Time Points |                          |                  |
|-----------------|----------------------------------|--------------------------|------------------|
|                 | Lag Phase                        | Exponential Growth Phase | Stationary Phase |
| 0.02            | Day 0                            | Day 8                    | Day 16           |
| 0.1             | Day 0                            | Day 11                   | Day 22           |
| 0.5             | Day 0                            | Day 12                   | Day 24           |

### 2.3. Speciation and Content Analysis of Extracellular and Intracellular As

To explore the impact of different  $\text{PO}_4^{3-}$  additions (0.02, 0.1 and 0.5 mg/L) on the metabolism of  $\text{iAs}^{\text{V}}$  in *M. aeruginosa* across the lag, exponential, and stationary growth phases, we collected all algal solutions at the end of the experiments for the  $\text{iAs}^{\text{V}}$  treatment groups (0.4, 0.6, 0.8, and 1 mg/L). The intra- and extracellular total As and As speciation were then assessed in both the media and algal cells. Approximately 0.005 g of freeze-dried algal samples underwent digestion with 2 mL  $\text{HNO}_3$  (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) at 120 °C [15]. Total As concentrations were determined using a hydride generation atomic fluorescence photometer (AFS-8230, Beijing Titan Co., Beijing, China). The actual concentration of the As stock solution was determined and verified using As quality control samples in drinking water, which was sourced from the China National Center for Standard Reference Materials. Any remaining As-contained wastes were collected in waste liquid barrels to be disposed of as hazardous waste by a qualified organization.

Arsenic speciation analysis in algal samples followed previously described methods [20]. In summary, freeze-dried cells were extracted with 1.75%  $\text{HNO}_3$  at 90 °C in three successive cycles. The supernatants were combined and filtered through 0.22  $\mu\text{m}$  nylon syringe filters for As species determination (i.e.,  $\text{iAs}^{\text{III}}$ , DMA, MMA, and  $\text{iAs}^{\text{V}}$ ), using high performance liquid chromatography coupled with HG-AFS (HPLC-HG-AFS, SA20, Beijing Titan Co., Beijing, China). The extraction method has demonstrated reliability, maintaining As speciation in *M. aeruginosa* within such treatments [21,22]. The recovery of As species ranged between 87% and 110% using 1.75%  $\text{HNO}_3$  extraction, indicating no significant loss or gain for  $\text{iAs}^{\text{III}}$  and  $\text{iAs}^{\text{V}}$  during the extraction process.

### 2.4. The Principles and Computation Methods for Various Factors

The algal cell densities varied under different growth conditions. Therefore, in this study, unit cell As concentrations were employed to assess both intra- and extracellular As concentrations [20]. The intracellular As content was computed using Equation (1), while the extracellular As content was determined using Equation (2) [23].

$$As_{in} = \frac{C_{As-in} \times V_{fix}}{X_{i+2} \times V_{sep}} \quad (1)$$

$$As_{ex} = \frac{C_{As-ex}}{X_{i+2}} \quad (2)$$

where

$As_{in}$  (fg /cell) denotes the intracellular As content per unit cell;

$C_{As-in}$  (fg/L) signifies the measured intracellular As content in the cells;

$V_{fix}$  (mL) indicates the final fixed volume;

$V_{sep}$  (mL) indicates the volume of separated algae;

$As_{ex}$  (fg /cell) indicates the extracellular As content per unit cell;

$C_{As-ex}$  (f/L) is the concentration of dissolved As in the growing media;

$X_{i+2}$  (cells/mL) signifies the algal cell density after 2 days of interaction with  $\text{iAs}^{\text{V}}$ .

However, the intra- and extracellular As concentrations are insufficient for evaluating the biological productivity of As through the use of algal cells [20]. This is because, after part of the exposed As was absorbed by the algae as  $\text{iAs}^{\text{V}}$ , a portion of it might undergo conversion, primarily to  $\text{iAs}^{\text{III}}$ , and also, to a lesser extent, to DMA and MMA [24]. Therefore, the present study was to further calculate the bioproductivities of As absorption ( $As_{ab}$ ), bioaccumulation (BCF), transformation ( $K_t$ ), and release ( $K_r$ ) from algae through the use of the following equations:

$$As_{ab} = As_{in-0} + As_{ex-2} + As_{ex-3} + As_{ex-4} \quad (3)$$

$$BCF = \frac{As_{in-0}}{As_{ex-1} + As_{ex-2} + As_{ex-3} + As_{ex-4}} \times 100\% \quad (4)$$

$$K_t = \frac{As_{ab} - As_{ex-1}}{As_{ab}} \times 100\% \quad (5)$$

$$K_r = \frac{As_{ex-2} + As_{ex-3} + As_{ex-4}}{As_{ab}} \times 100\% \quad (6)$$

where

$As_{ab}$  represents the As absorption per cell (fg/cell), encompassing both intra- and extracellular As, but excluding the  $iAs^V$  form;

$K_t$  denotes the transformation rate;

$K_r$  denotes the releasing rate;

$As_{in-0}$ ,  $As_{in-1}$ ,  $As_{in-2}$ ,  $As_{in-3}$ , and  $As_{in-4}$  denote the intracellular total As,  $iAs^V$ ,  $iAs^{III}$ , DMA, and MMA per unit cell concentration, respectively;

$As_{ex-1}$ ,  $As_{ex-2}$ ,  $As_{ex-3}$ , and  $As_{ex-4}$  represent the extracellular dissolved  $iAs^V$ ,  $iAs^{III}$ , DMA, and MMA per unit cell concentration, respectively.

Notably, the extracellular  $iAs^V$ , designated as  $As_{ex-1}$ , is not considered in Equation (5) since it can be viewed as the remaining unchanged As initially added to the growth media.

### 2.5. Statistical Analysis

Statistical analyses were conducted using IBM-SPSS 23 software. A one-way ANOVA with a Duncan test was employed to assess the inter and intra differences in the algae across various factors, i.e., intra- and extracellular As concentrations ( $As_{in}$ ,  $As_{ex}$ ), as well as As absorption ( $As_{ab}$ ), bioaccumulation (BCF), transformation ( $K_t$ ), and release ( $K_r$ ). The results were expressed as mean  $\pm$  standard deviation.

## 3. Results and Discussion

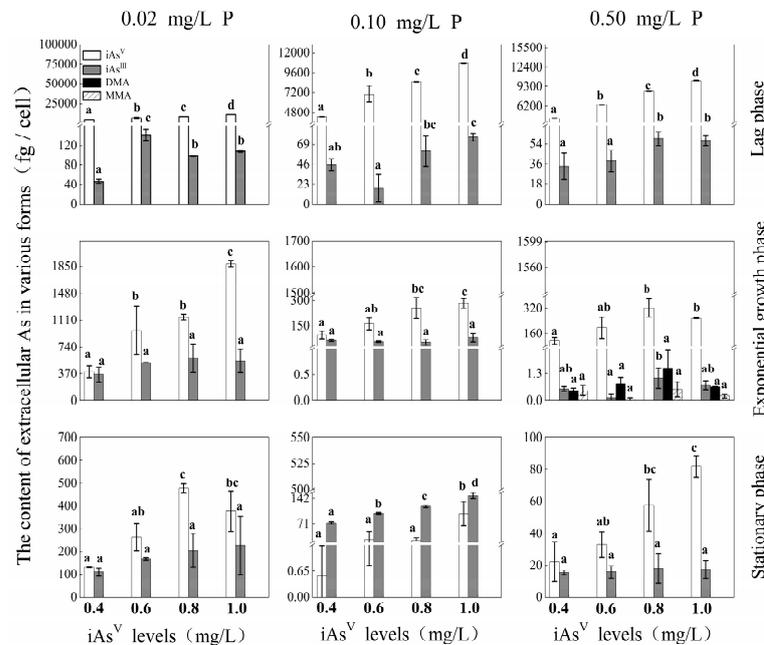
### 3.1. Effects of P and $iAs^V$ Additions on Extracellular As Species and Content at Different Growth Phases

$iAs^V$  maintained its dominance as the primary As species during the lag and exponential growth phases in the media (Figure 1). However,  $iAs^{III}$  dominated during the stationary growth phase with 0.1 mg/L P, intensifying As-related ecological risks [25]. Additionally, the concentration of  $iAs^{III}$  in the media exhibited a significant negative correlation with the P level during the exponential growth phase, suggesting that P could inhibit extracellular  $iAs^{III}$  production to a certain extent, with this inhibitory effect tending to increase with higher P concentrations. This inhibition may be attributed to the crucial role of P as an essential nutrient for algal growth, influencing physiological metabolic processes such as the algal dissimilation reduction process [26,27]. Furthermore, trace amounts of organic As species, including DMA and MMA, were observed during the exponential growth phase with 0.5 mg/L P. This indicates that a high concentration of P could promote the transformation of DMA and MMA via *M. aeruginosa*. This finding was consistent with previous research, showing that As methylation induced by *M. aeruginosa* was influenced by different P levels and growth phases in the aquatic environment [28].

### 3.2. Effects of P and $iAs^V$ Additions on Intracellular As Species and Content at Different Growth Phases

Due to the structural resemblance between  $iAs^V$  and P, their uptake in algal cells was believed to occur via similar transporters. Consequently, an increased trend of P levels was expected to result in a reduction in As accumulation in algal cells [29]. As depicted in Figure 2, the speciation and total content of As in algal cells after being exposed to  $iAs^V$  exhibited significant differences across three P levels during various growth phases. At the lag phase with three P levels (0.02, 0.1, and 0.5 mg/L), the levels of intracellular  $iAs^{III}$  and  $iAs^V$  showed no significant linear relationships with  $iAs^V$  additions. During the exponential phase with a 0.02 mg/L P level, both intracellular  $iAs^{III}$  and  $iAs^V$  exhibited significant positive linear relationships with  $iAs^V$  additions ( $p < 0.05$ ,  $R^2 = 0.813$  and  $0.842$ );

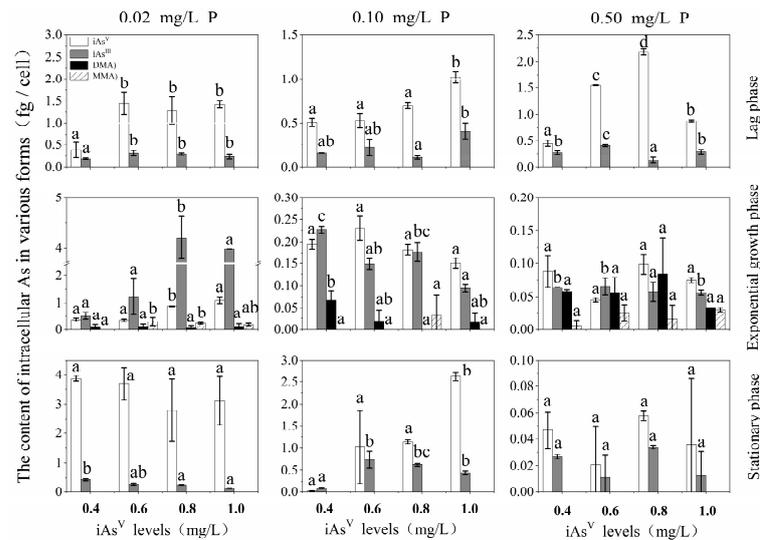
however, at 0.1 and 0.5 mg/L of P, intracellular  $iAs^V$  remained unchanged with  $iAs^V$  additions ( $p > 0.05$ ), and intracellular  $iAs^{III}$  did not show any significant linear relationships with  $iAs^V$  additions ( $p < 0.05$ ,  $R^2 = 0.648$  and  $0.282$ ). Additionally, intracellular MMA and DMA showed no significant changes with varying  $iAs^V$  levels ( $p > 0.05$ ) at all P levels. At the stationary phase, intracellular  $iAs^{III}$  and  $iAs^V$  exhibited a significant negative linear relationship with  $iAs^V$  additions at 0.02 mg/L P ( $p < 0.05$ ), but a positive relationship at 0.1 mg/L P ( $p < 0.05$ ).



**Figure 1.** The contents ( $1 \text{ fg} = 10^{-15} \text{ g}$ ) and species of extracellular As in different growth periods with various P and  $iAs^V$  levels. Note: different lowercase letters indicate that there was a significant difference in the content of the same form of As between different  $iAs^V$  levels at same growth phase and P level ( $p < 0.05$ ).

In this study, higher As content in *M. aeruginosa* was observed under 0.02 mg/L phosphate during the stationary growth phase. Wang et al. investigated the toxicity and bioaccumulation kinetics of arsenate in algae under phosphate-enriched (+P) and limited (−P) conditions, finding that algae exhibit maximum As uptake under low P conditions [30]. This is because there is no extracellular phosphate to compete with  $iAs^V$  for phosphate transport systems. The content of  $iAs^V$  in *M. aeruginosa* was higher than  $iAs^{III}$  during incubation following the algal exposure to  $iAs^V$  during the lag and stationary growth phases, especially at the lower P addition (0.02 mg/L), indicating that P could influence the transformation of As species in *M. aeruginosa*. Ding et al. have also reported that a high concentration of intracellular  $iAs^V$ , resulting from  $iAs^V$  intake, was found in a reservoir with P restrictions [27].

Additionally, cellular  $iAs^{III}$  concentrations in lower P-treated *M. aeruginosa*, resulting from the reductive metabolism of algae following the intake of  $iAs^V$ , were much higher than those in higher P-treated cells during the exponential growth phase, indicating that P could influence the transformation of As in *M. aeruginosa*. Studies have demonstrated that phosphate is a crucial environmental factor, influencing As metabolism in algae because of its structural similarity to  $iAs^V$ , which allows it to compete with  $iAs^V$  for uptake via the designated phosphate transporters in the cell membrane [31]. Our results revealed that the presence of phosphate could inhibit intracellular As content by decreasing the uptake of  $iAs^{III}$  or  $iAs^V$ , thus mitigating As toxicity to *M. aeruginosa*. This finding aligns with other studies that reported a noticeable decrease in  $iAs^V$  uptake by *Chlorella salina* and *Chlamydomonas reinhardtii* [32–34].



**Figure 2.** The contents ( $1 \text{ fg} = 10^{-15} \text{ g}$ ) and species of intracellular As in different growth periods with various P and  $i\text{As}^{\text{V}}$  levels. Note: different lowercase letters indicate that there is a significant difference in the content of the same  $i\text{As}^{\text{V}}$  forms between different  $i\text{As}^{\text{V}}$  levels at the same growth period and phosphorus level ( $p < 0.05$ ).

### 3.3. Effects of P and $i\text{As}^{\text{V}}$ Additions on As Metabolism at Different Growth Phases

Significant variations in As absorption ( $\text{As}_{\text{ab}}$ ), bioaccumulation (BCF), and release metabolized by *M. aeruginosa* were observed at different P and  $i\text{As}^{\text{V}}$  additions across various growth phases (Tables 3–5). The As absorption ( $\text{As}_{\text{ab}}$ ) showed a positive relationship with  $i\text{As}^{\text{V}}$  levels only during the lag phase. In contrast to the lag phase, As absorption by *M. aeruginosa* was not markedly affected by  $i\text{As}^{\text{V}}$  additions during the exponential and stationary growth phases. As absorption ( $\text{As}_{\text{ab}}$ ) decreased with increased P addition;  $\text{As}_{\text{ab}}$  was namely restricted by P. For instance, at the exponential growth phase, the absorption values of As were  $5.065 \times 10^{-7}$ ,  $6.449 \times 10^{-8}$ , and  $1.992 \times 10^{-9} \text{ g/cell}$  under low, medium, and high P levels, respectively. At the stationary phase, the corresponding absorption values were  $1.804 \times 10^{-7}$ ,  $1.123 \times 10^{-7}$ , and  $1.669 \times 10^{-8} \text{ g/cell}$ . The presence of P in the media potentially induced the increasing P demand and utilization of algal cells, leading to altered  $i\text{As}^{\text{V}}$  metabolism levels.  $i\text{As}^{\text{V}}$  was found to alter the photosynthetic apparatus and reduce the ability to retain P in *M. aeruginosa* under P limitation conditions [35], thus leading to higher As absorption.

The As bioaccumulation exhibited variation across different growth phases, with 0.04%, 0.3%, and 2% at the lag, exponential, and stationary phases, respectively. This suggests that the capacity of algal As enrichment positively correlates with algal reproduction. Simultaneously, both lower and higher levels of P (0.02 and 0.5 mg/L) were found to significantly decrease As accumulation in algae cells during the exponential and stationary growth phases (Table 4). This phenomenon can be explained by the differences of P consumption for the synthesis of various metabolites and P storage.

The biotransformation of total As did not show significant changes with increasing P additions from 0.02 mg/L to 5 mg/L under all  $i\text{As}^{\text{V}}$  levels (Table 6). This indicated that increasing P levels could not cause a difference in total As conversion, aligning with a previous study that found similar total  $i\text{As}^{\text{V}}$  biotransformation amounts per *M. aeruginosa* cell among different P conditions [28]. However, during the exponential growth phase, intra- and extracellular  $i\text{As}^{\text{III}}$  content decreased with increasing P levels from 0.02 mg/L to 0.5 mg/L for cells, coupled with a slight increase in MMA and DMA biotransformation (Figures 1 and 2). This indicated that increasing P could inhibit  $i\text{As}^{\text{III}}$  transformation, while promoting methylated As transformation. This was partly attributed to the positive roles of P as one of the main elements of ATP or NADP, both important driving forces for facilitating the accumulation of metabolites [36].

**Table 3.** The As absorption by cells in different growth phases; P and iAs<sup>V</sup> levels (fg/cell).

| As Addition (mg/L) | P Levels (mg/L)              |                             |                             |                               |                             |                           |                               |                              |                            |
|--------------------|------------------------------|-----------------------------|-----------------------------|-------------------------------|-----------------------------|---------------------------|-------------------------------|------------------------------|----------------------------|
|                    | Lag Growth Phase             |                             |                             | Exponential Growth Phase      |                             |                           | Stationary Growth Phase       |                              |                            |
|                    | 0.02                         | 0.1                         | 0.5                         | 0.02                          | 0.1                         | 0.5                       | 0.02                          | 0.1                          | 0.5                        |
| 0.4                | 46.98 ± 3.11 <sup>Ab</sup>   | 49.33 ± 0.84 <sup>Aab</sup> | 34.46 ± 11.83 <sup>Aa</sup> | 362.07 ± 103.30 <sup>Ba</sup> | 65.47 ± 6.33 <sup>Aa</sup>  | 1.70 ± 0.07 <sup>Aa</sup> | 113.95 ± 17.87 <sup>Ca</sup>  | 72.96 ± 2.99 <sup>Ba</sup>   | 15.43 ± 1.31 <sup>Aa</sup> |
| 0.6                | 143.23 ± 11.76 <sup>Bc</sup> | 18.64 ± 15.48 <sup>Aa</sup> | 40.28 ± 9.55 <sup>Aa</sup>  | 525.42 ± 1.83 <sup>Ca</sup>   | 57.06 ± 3.58 <sup>Ba</sup>  | 1.15 ± 0.08 <sup>Aa</sup> | 170.54 ± 5.24 <sup>Ca</sup>   | 102.26 ± 3.43 <sup>Bb</sup>  | 15.82 ± 3.91 <sup>Aa</sup> |
| 0.8                | 100.12 ± 1.70 <sup>Bb</sup>  | 64.43 ± 23.88 <sup>Ab</sup> | 60.64 ± 6.03 <sup>Ab</sup>  | 589.50 ± 201.76 <sup>Ba</sup> | 54.63 ± 14.77 <sup>Aa</sup> | 3.36 ± 1.86 <sup>Aa</sup> | 207.91 ± 72.10 <sup>Ba</sup>  | 120.52 ± 3.15 <sup>ABc</sup> | 18.10 ± 9.30 <sup>Aa</sup> |
| 1                  | 109.43 ± 2.25 <sup>Bb</sup>  | 70.34 ± 13.50 <sup>Ab</sup> | 57.94 ± 4.32 <sup>Ab</sup>  | 549.20 ± 162.99 <sup>Ba</sup> | 80.82 ± 25.13 <sup>Aa</sup> | 1.76 ± 0.15 <sup>Aa</sup> | 229.32 ± 126.03 <sup>Aa</sup> | 153.61 ± 8.88 <sup>Ad</sup>  | 17.43 ± 5.55 <sup>Aa</sup> |

Note: Different lowercase letters indicate that there is a significant difference in the absorption of As at the same growth phase and P level ( $p < 0.05$ ). Different capital letters indicate that there is a significant difference in the absorption of As between different P levels at the same growth phase and iAs<sup>V</sup> level ( $p < 0.05$ ).

**Table 4.** The BCF of As by cells under different growth phases; P and iAs<sup>V</sup> levels (%).

| As Addition (mg/L) | P Levels (mg/L)            |                    |                    |                           |                           |                           |                           |                            |                           |
|--------------------|----------------------------|--------------------|--------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|---------------------------|
|                    | Lag Growth Phase           |                    |                    | Exponential Growth Phase  |                           |                           | Stationary Growth Phase   |                            |                           |
|                    | 0.02                       | 0.1                | 0.5                | 0.02                      | 0.1                       | 0.5                       | 0.02                      | 0.1                        | 0.5                       |
| 0.4                | 0.01 ± 0.01 <sup>Aa</sup>  | 0.01 <sup>Ab</sup> | 0.01 <sup>Ab</sup> | 0.09 ± 0.02 <sup>Aa</sup> | 0.26 ± 0.04 <sup>Bc</sup> | 0.17 <sup>Ab</sup>        | 1.68 ± 0.07 <sup>Bb</sup> | 0.13 <sup>Aa</sup>         | 0.17 ± 0.01 <sup>Ab</sup> |
| 0.6                | 0.03 <sup>Bb</sup>         | 0.01 <sup>Aa</sup> | 0.03 <sup>Bd</sup> | 0.10 ± 0.04 <sup>Aa</sup> | 0.14 ± 0.04 <sup>Ab</sup> | 0.07 ± 0.01 <sup>Aa</sup> | 0.90 ± 0.35 <sup>Aa</sup> | 1.29 ± 0.73 <sup>Ab</sup>  | 0.10 <sup>Aa</sup>        |
| 0.8                | 0.02 ± 0.01 <sup>ABa</sup> | 0.01 <sup>Aa</sup> | 0.02 <sup>Bc</sup> | 0.29 ± 0.01 <sup>Cc</sup> | 0.10 <sup>Bab</sup>       | 0.05 ± 0.02 <sup>Aa</sup> | 0.41 ± 0.23 <sup>Aa</sup> | 1.21 ± 0.13 <sup>Bab</sup> | 0.10 ± 0.04 <sup>Aa</sup> |
| 1                  | 0.01 <sup>Ba</sup>         | 0.01 <sup>Bb</sup> | 0.01 <sup>Aa</sup> | 0.21 ± 0.01 <sup>Bb</sup> | 0.05 ± 0.02 <sup>Aa</sup> | 0.05 <sup>Aa</sup>        | 0.49 ± 0.22 <sup>Aa</sup> | 1.22 ± 0.18 <sup>Ab</sup>  | 0.08 <sup>Aa</sup>        |

Note: Different lowercase letters indicate that there are significant differences in the enrichment coefficients of As between different iAs<sup>V</sup> levels at the same growth phase and P level ( $p < 0.05$ ). Different capital letters indicate that there are significant differences in the enrichment coefficients of As between the same growth phase and different P treatment conditions at the same iAs<sup>V</sup> level ( $p < 0.05$ ).

**Table 5.** The As transformation by cells under different growth phases; P and iAs<sup>V</sup> levels (%).

| As Addition (mg/L) | P Levels (mg/L)            |                             |                            |                            |                            |                            |                            |                             |                            |
|--------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|
|                    | Lag Growth Phase           |                             |                            | Exponential Growth Phase   |                            |                            | Stationary Growth Phase    |                             |                            |
|                    | 0.02                       | 0.1                         | 0.5                        | 0.02                       | 0.1                        | 0.5                        | 0.02                       | 0.1                         | 0.5                        |
| 0.4                | 99.43 ± 0.50 <sup>Aa</sup> | 99.14 ± 0.12 <sup>Aa</sup>  | 98.83 ± 0.29 <sup>Ab</sup> | 99.94 ± 0.07 <sup>Aa</sup> | 99.77 <sup>Ab</sup>        | 95.21 ± 2.80 <sup>Aa</sup> | 96.75 ± 0.43 <sup>Aa</sup> | 99.98 ± 0.03 <sup>Bb</sup>  | 99.72 ± 0.15 <sup>Ba</sup> |
| 0.6                | 98.86 ± 0.28 <sup>Aa</sup> | 74.58 ± 42.08 <sup>Aa</sup> | 96.30 ± 1.11 <sup>Aa</sup> | 99.97 ± 0.01 <sup>Aa</sup> | 99.69 ± 0.04 <sup>Aa</sup> | 98.40 ± 0.37 <sup>Aa</sup> | 97.93 ± 0.46 <sup>Aa</sup> | 99.06 ± 0.99 <sup>Aab</sup> | 99.72 <sup>Aa</sup>        |
| 0.8                | 98.84 ± 0.60 <sup>Ba</sup> | 98.97 ± 0.27 <sup>Ba</sup>  | 96.64 ± 0.29 <sup>Aa</sup> | 99.87 ± 0.06 <sup>Ba</sup> | 99.76 ± 0.01 <sup>Bb</sup> | 98.16 ± 0.37 <sup>Aa</sup> | 98.55 ± 1.14 <sup>Aa</sup> | 99.08 ± 0.09 <sup>Aab</sup> | 99.71 ± 0.10 <sup>Aa</sup> |
| 1                  | 98.81 ± 0.27 <sup>Aa</sup> | 98.62 ± 0.29 <sup>Aa</sup>  | 98.82 ± 0.05 <sup>Ab</sup> | 99.82 ± 0.11 <sup>Ba</sup> | 00.87 ± 0.03 <sup>Bc</sup> | 97.59 ± 0.42 <sup>Aa</sup> | 98.37 ± 1.41 <sup>Aa</sup> | 98.30 ± 0.04 <sup>Aa</sup>  | 99.55 <sup>Aa</sup>        |

Note: different lowercase letters indicate that there is a significant difference in transformation rate between different iAs<sup>V</sup> levels at the same growth phase and P level ( $p < 0.05$ ). Different capital letters indicate that there is a significant difference in the transformation rate of As between different P levels at the same growth phase and iAs<sup>V</sup> level ( $p < 0.05$ ).

**Table 6.** The release rate of As by cells under different growth phases; P and iAs<sup>V</sup> levels (%).

| As Addition<br>(mg/L) | P levels (mg/L)            |                             |                            |                            |                             |                            |                            |                             |                            |
|-----------------------|----------------------------|-----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|
|                       | Lag Growth Phase           |                             |                            | Exponential Growth Phase   |                             |                            | Stationary Growth Phase    |                             |                            |
|                       | 0.02                       | 0.1                         | 0.5                        | 0.02                       | 0.1                         | 0.5                        | 0.02                       | 0.1                         | 0.5                        |
| 0.4                   | 99.11 ± 0.54 <sup>Aa</sup> | 98.81 ± 0.14 <sup>Aa</sup>  | 98.13 ± 0.55 <sup>Ab</sup> | 99.81 ± 0.01 <sup>Bb</sup> | 99.37 ± 0.04 <sup>Ba</sup>  | 88.76 ± 1.74 <sup>Aa</sup> | 96.41 ± 0.42 <sup>Aa</sup> | 99.87 <sup>Bb</sup>         | 99.58 ± 0.18 <sup>Ba</sup> |
| 0.6                   | 98.67 ± 0.26 <sup>Aa</sup> | 65.58 ± 56.79 <sup>Aa</sup> | 95.50 ± 1.22 <sup>Aa</sup> | 99.70 ± 0.19 <sup>Ba</sup> | 99.46 ± 0.10 <sup>Bab</sup> | 88.22 ± 4.14 <sup>Aa</sup> | 97.80 ± 0.52 <sup>Aa</sup> | 98.35 ± 1.18 <sup>Aab</sup> | 99.61 <sup>Aa</sup>        |
| 0.8                   | 98.57 ± 0.53 <sup>Ba</sup> | 98.84 ± 0.45 <sup>Ba</sup>  | 96.58 ± 0.39 <sup>Aa</sup> | 99.10 ± 0.21 <sup>Ba</sup> | 99.40 ± 0.25 <sup>Bab</sup> | 94.73 ± 0.13 <sup>Aa</sup> | 98.47 ± 1.18 <sup>Aa</sup> | 98.58 ± 0.08 <sup>Aab</sup> | 99.58 ± 0.09 <sup>Aa</sup> |
| 1                     | 98.66 ± 0.26 <sup>Aa</sup> | 98.03 ± 0.69 <sup>Aa</sup>  | 98.54 ± 0.10 <sup>Ab</sup> | 99.04 ± 0.32 <sup>Ba</sup> | 99.78 ± 0.01 <sup>Bb</sup>  | 92.11 ± 0.90 <sup>Aa</sup> | 98.34 ± 1.41 <sup>Aa</sup> | 98.04 ± 0.06 <sup>Aa</sup>  | 99.42 <sup>Aa</sup>        |

Note: different lowercase letters indicate that there is a significant difference in the release rate of As at the same growth phase and P level ( $p < 0.05$ ). Different capital letters indicate that there is a significant difference in the release rate of As between P levels at the same growth phase and iAs<sup>V</sup> level ( $p < 0.05$ ).

The As release rate ( $K_r$ ) surpassed 96% during the lag phase, exhibiting non-significant changes with varying As and P levels. In the exponential period, the release rate exceeded 85%, displaying a negative correlation with P, while in the stationary growth phase, the release rate exceeded 96%, indicating a positive correlation with P. These findings suggested that the changes of the release rate mirror those in the transformation rate, implying a relationship between As release and As transformation. Research had indicated that P indirectly influenced As release by affecting As transformation [9], establishing no direct relationship between As release and P levels. Consequently, P indirectly affected As release and promoted As efflux.

#### 4. Conclusions

In this study, it was observed that  $iAs^V$  addition significantly promoted the As metabolism of *M. aeruginosa* in media with 0.1 mg/L of P. Conversely, the metabolism of  $iAs^V$  was inhibited in lower (0.02 mg/L) or higher P (0.5 mg/L) additions. The influence of P and  $iAs^V$  additions on the absorption ( $As_{ab}$ ), bioaccumulation (BCF), transformation, and release of As from *M. aeruginosa* was evident, and this impact was contingent on the initial P status of the algal cells at various growth phases.

Moreover,  $iAs^V$  emerged as the predominant species in algal cells in the M11 media under different P and  $iAs^V$  additions, while DMA and MMA exhibited a significant increase during the exponential growth phase with 0.5 mg/L of P in media. The highest intracellular As content was observed at the 0.02 mg/L P addition for algal cells. The initial P status of algal cells played a crucial role in  $iAs^V$  metabolism, leading to distinct fates, even under the same  $iAs^V$  addition. Furthermore, the highest amount of  $iAs^V$  metabolized by *M. aeruginosa* was observed under conditions of low levels of P during the stationary growth phase. A higher level of P in the media accelerated  $iAs^V$  biotransformation into  $iAs^{III}$ , DMA, and MMA by *M. aeruginosa* during the exponential growth phase. Increasing  $iAs^V$  levels in media under a lower P level promoted  $iAs^V$  biotransformation for algal cells during the exponential growth phase, especially to  $iAs^{III}$  and MMA, but not to DMA. These findings provide insights into As metabolism under different  $iAs^V$  and  $PO_4^{3-}$  levels with algal blooms in freshwater ecosystems.

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