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Biosynthesis and Release of Methylarsenic Compounds During the Growth of Freshwater Algae

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Abstract

Arsenic transformations by freshwater algae have been studied under laboratory conditions. By the use of a new analytical method, we identified methylarsenic(III) species in the growth medium of green-alga *Closterium aciculare* incubated under axenic conditions. The arsenate concentration in the experimental medium began to decrease just after inoculation, and the levels of arsenite and methylarsenicals increased with the growth of *C. aciculare*. Initially, most of the arsenate decrement was converted into arsenite, which peaked in concentration during the exponential phase. Methylarsenicals accumulated rapidly in the stationary phase. DMAA(V) production was enhanced when the ratio of phosphate to arsenate decreased in the culture medium. The levels of DMAA(V) increased continuously toward the end of the experiment. On the other hand, methylarsenic(III) species remained relatively steady during the stationary phase. Methylarsenic(III) species accounted for 0-35% of methylarsenicals. These results suggest that arsenite and methylarsenicals (containing methylarsenic(III) species) are supplied by phytoplankton, and serve as evidence of the origin of methylarsenic(III) species in natural waters.

Keywords: arsenic speciation; methylarsenicals; phytoplankton; methylarsenic(III) species; biotransformation

1. Introduction

Arsenic is taken up by organisms and can undergo reduction and methylation to form a variety of dissolved forms in natural waters. In eutrophic regions, the proportion of arsenite and methylarsenicals is significantly large, while arsenate is thermodynamically the most stable form of arsenic in oxic environments (Andreae, 1986; Cullen and Reimer, 1989; Francesconi and Edmonds, 1993; Francesconi and Edmonds, 1997). Field investigations in freshwater demonstrated that methylarsenicals dominated over other organoarsenicals, and that methylarsenic concentrations were increased in surface water and above the sediment (Andreae 1978; Anderson and Bruland 1991; Kuhn and Sigg 1993; Hasegawa 1997; Sohrin et al. 1997a). The distribution of arsenic species indicates that the occurrence of organoarsenic compounds is correlated with biological activity in the water column. Methylarsenicals in natural waters are expected to be produced directly by phytoplankton and bacteria, and to result from the degradation of biological materials. Methylation of arsenate to monomethylated and dimethylated forms occurs in various algal cultures. The production of methylarsenic(V) species by marine algae (Andreae and Klumpp, 1979; Sanders and Windom, 1980; Sanders and Vermersch, 1982; Sanders and Riedel, 1993) and freshwater algae (Baker et al., 1983; Nissen and Benson, 1982; Maeda et al., 1987; Maeda et al., 1992) has been reported.

Recently, we developed a new hydride generation technique that is sensitive enough to determine methylarsenic(III) species, monomethylarsonous acid [$CH_3As(OH)_2$; MMAA(III)] and dimethylarsinous acid [$(CH_3)_2As(OH)$; DMAA(III)], and methylarsenic(V) species, methylarsonic acid [$CH_3AsO(OH)_2$; MMAA(V)] and dimethylarsinic acid [$(CH_3)_2AsO(OH)$; DMAA(V)], separately (Hasegawa et al., 1994).

In studies in freshwater and coastal seawater, the concentration of methylarsenic(III) species was one or two orders of magnitude lower than that of methylarsenic(V) species because of a short half-life in the oxic environment (Hasegawa, 1996; Hasegawa, 1997; Sohrin et al. 1997a). However, methylarsenic(III) distribution changed seasonally, perhaps due to the seasonal blooms of phytoplankton.

Here we report a series of algal culture experiments designed to demonstrate the methylation of arsenic and the production of methylarsenic(III) species in freshwater. The changes in the species distribution of arsenic were measured in axenic culture media in which arsenate and nutrient concentrations were controlled at different levels. This is the first report of methylarsenic(III) species being found and measured in phytoplankton cultures. The information would provide for a better interpretation of arsenic biogeochemistry in the environment.

2. Materials and methods

2.1 Culture experiments

Clonal axenic strains of *Closterium aciculare*, *Aulacoseira granulata* and *Pediastrum biwae*, isolated by Dr. H. Nakahara (Graduate School of Agriculture, Kyoto University) from Lake Biwa, Japan, were used throughout the study. Before the experiments, the clonal cultures were maintained in the same medium for 1-2 week until cells were at an exponential phase of growth. Experimental cultures were grown at 25 °C under a 12:12 h L/D photoperiod at a light intensity of 50 μE m^{-2} s^{-1} provided by cool white fluorescent lights. The axenic nature was verified frequently by DAPI direct straining and

examination under an epifluorescent microscope (Bohlool et al., 1980).

Five-liter capacity glass vessels containing 4 liters of sterilized CT medium (Watanabe and Ichimura, 1977), modified by reducing the concentration of nitrate and phosphate (added as KH_2PO_4 instead of sodium glycerophosphate) to encourage uptake of arsenate, were incubated with acclimated exponential phase cells. Three different conditions of arsenate and nutrient concentrations were tested (Table 1). On examination for the effect of arsenic species on the growth of *C. aciculare* in the modified CT medium (containing 11 μ mol L^{-1} of phosphate), the populations of *C. aciculare* at steady state were unaffected by arsenite, arsenate, MMAA(V) and DMAA(V) at less than 60 μmol L^{-1}. Sterilized air was introduced from the bottom of the vessel and the contents were gently mixed. At the beginning of the experiment, the initial cell density was 20 cells mL^{-1}. Cultures were grown for a period of 3-5 weeks. Phytoplankton growth was followed by measuring spectrophotometrically on a UV-VIS spectrophotometer at 750 nm, and correlated with an established cell density-to-absorbance ratio to estimate cell number. Measurements of cell number were made by counting directly by microscope.

2.2 Analytical procedures

Samples were taken by blocking an aeration exhaust tube of the vessel, thereby pressurizing the vessel and forcing the samples of the culture through a sample tube. The samples were passed through 0.45 μm filters (Millipore, HA) under low vacuum pressure (< 30 mmHg). This procedure was performed under nitrogen gas in order to prevent oxidation of arsenic(III) species.

Arsenic species were measured by an improved hydride generation technique*

(Hasegawa et al., 1994). The analytical and operating conditions were as described previously. Arsenic(III) species were extracted from 200 ml samples with diethylammonium diethyldithiocarbamate into carbon tetrachloride. After back-extraction, inorganic and methylated forms were measured by hydride generation with liquid nitrogen trapping, chromatographic separation and atomic absorption detection (HG-AAS; Braman et al., 1977; Andreae, 1977; Andreae, 1979). The concentrations of arsenic(III+V) species were measured from 50 ml samples directly using the HG-AAS, and the concentrations of arsenic(V) species were calculated from the difference between arsenic(III+V) species and arsenic(III) species. Minimum detectable concentrations were 0.027, 0.043 and 0.030 nmol L^{-1} for arsenite, MMAA(III) and DMAA(III), and 0.11, 0.18 and 0.12 nmol L^{-1} for arsenate, MMAA(V) and DMAA(V), respectively. Other organoarsenic compounds were measured as the ultraviolet-labile fraction by Howard's method (Howard, 1989). Total arsenic concentration was determined after the alkaline persulfate oxidation procedure which is a modification of the method described by Le et al. (1992). Phosphate was determined by phosphomolybdate blue spectrophotometry. For nitrate analysis, nitrate was reduced with hydrazine, diazotized and determined spectrophotometrically (Kitamura, 1982).

* In this study, we examined hydride-reducible organoarsenic species in water samples. These compounds were "mono-" and "dimethylarsenic", and converted to mono- and dimethylarsine, respectively, by sodium tetrahydroborate. There is general agreement that the majority of mono- and dimethylarsenic species are MMAA(V) and DMAA(V) in natural waters. Recently, it was reported that dissolved dimethylarsenic closely resembled DMAA(V) in estuarine waters (Howard et al., 1999). Therefore, the terms MMAA(V)

and DMAA(V) were used instead of "mono-" and "dimethylarsenic" in this paper.

3. Results and Discussion

3.1 Arsenic speciation changes in phosphate-deficient arsenic-high cultures of C. aciculare

The green algae *Closterium aciculare* is resistant to the inhibitory effect of arsenic, and grew well in 0.1-200 μmol L^{-1} arsenate. *C. aciculare* produced arsenite and methylarsenicals in the presence of arsenate. The concentrations of arsenic and nutrient were measured during exponential and stationary phases of growth (Figs. 1-3). Under phosphate-deficient arsenic-high conditions, the growth of *C. aciculare* was followed by decreases in phosphate during the exponential phase (Fig. 1). *C. aciculare* had attained a density of 3.6 x 10^4 cells mL^{-1} by day 14, then showed relatively steady growth toward the end of the experiment. The initial level of phosphate was 19 μmol L^{-1} which had decreased to 1.8 μmol L^{-1} by the end of the exponential phase. The concentration of arsenate, 12 μmol L^{-1} initially, decreased with phytoplankton growth. The As(III) concentration reached a maximum of 270 nmol L^{-1} as cell numbers increased exponentially, but decreased to between 30 nmol L^{-1} and 50 nmol L^{-1} during the stationary phase. Methylarsenic species began to appear at the end of the exponential phase. Methylarsenic(V) species (DMAA(V) and MMAA(V)) were characterized by rapid increases in the stationary phase, followed by a gradual increase toward the end of the experiment. During the stationary phase, DMAA(V) increased from 1.0 μmol L^{-1} to 1.9 μmol L^{-1}, and was an order of magnitude more abundant than MMAA(V) (61-120 nmol L^{-1}). On the other hand, methylarsenic(III) species (DMAA(III) and MMAA(III))

consisted of comparable amounts of monomethylated and dimethylated forms. DMAA (III) and MMAA(III) showed maximum concentrations of 4.6 nmol L^{-1} and 7.3 nmol L^{-1} at the initial stage of the stationary growth, and then remained steady at 1.4-2.0 nmol L^{-1} and 3.2-4.0 nmol L^{-1} during the stationary phase, respectively. Ultraviolet-labile fractions of organoarsenicals were below the detection limit (<0.11 nmol L^{-1}) throughout the experiments, and the concentrations of total dissolved arsenic agreed well with the sums of inorganic and methylated arsenic concentrations in each culture medium.

We also observed the species distribution in phosphate-deficient arsenic-high culture medium every 2 hours for 24 hours, and estimated the rates of uptake of phosphate and production of arsenic species (Table 2). *C. aciculare* grew from 3.7 x 10^3 to 6.2 x 10^3 cells mL^{-1} during the 24-hour experiment. The concentrations of phosphate and arsenic species changed linearly with time during the light and dark phase. The production and uptake rates (k) were, therefore, estimated by $k = (C_{t1}-C_{t2})/(t_1-t_2)$, where C_{t1} and C_{t2} are the concentrations at times t_1 and t_2. The production rates of arsenite and DMAA(V) remained constant both in the light and dark phase. The production rates of DMAA(III) were associated with the L/D cycles, and were low during the light phase.

3.2 Arsenic speciation changes in phosphate-deficient arsenic-low cultures of C. aciculare

Figure 2 shows the change in concentrations of arsenic species in the *C. aciculare* cultures under phosphate-deficient arsenic-low conditions. The initial arsenate concentration (10 nmol L^{-1}) was in the more commonly observed range of 5-15 nmol L^{-1} in Lake Biwa, and was three orders of magnitude lower than that in the arsenic-high medium. Arsenate was replaced initially by arsenite during the exponential phase and then

by DMAA(V) at the initial stage of stationary growth, which was similar to the result of the previous experiment in phosphate-deficient arsenic-high cultures (Fig. 1). DMAA(V) increased from 0.83 nmol L^{-1} to 4.4 nmol L^{-1} during the stationary phase. MMAA(V), MMAA(III) and DMAA(III) were below the detection limit (< 0.11 nmol L^{-1}) in the phosphate-deficient arsenic-low medium. Although the densities of *C. aciculare* were comparable to those of the arsenic-high medium, the levels of arsenite and methylarsenicals were lower.

3.3 Arsenic speciation changes in nutrient-deficient arsenic-low cultures of C. aciculare

In Fig. 3, the changes to arsenic and nutrient in the *C. aciculare* cultures under nutrient-deficient arsenic-low conditions are demonstrated. The levels of arsenate, phosphate and nitrate were similar to those in Lake Biwa (Sohrin et al., 1997b). The cell densities of *C. aciculare* were reduced compared with in the preceding experiments. Arsenite showed a maximum concentration of 4.5 nmol L^{-1} during the exponential phase, and then remained steady at 2.1-2.3 nmol L^{-1} during the stationary phase. DMAA(V) and DMAA(III) were found in the range of 0.45-0.63 nmol L^{-1} and 0.05-0.17 nmol L^{-1}, respectively, during the stationary phase. The concentrations of DMAA(III) are consistent with the observed values of 0.1-0.2 nmol L^{-1} methylarsenic(III) during the natural phytoplankton blooms of the southern basin in Lake Biwa (Sohrin et al, 1995; Sohrin et al. 1997a; Hasegawa 1997).

3.4 Distribution of arsenic species in the cultures of C. aciculare

Arsenate was converted to arsenite and methylarsenicals by *C. aciculare* (Figs. 1-3).

The species distribution of arsenic during the stationary phase (after phosphate depletion) is summarized in Table 3. Main species were arsenite and DMAA(V), and minor species were MMAA(V), DMAA(III) and MMAA(III) in the cultures of *C. aciculare*. The proportion of arsenite and DMAA(V) in the media accounted for <0.1-27% and 4.3-43%, respectively, of initial arsenate during the stationary phase. MMAA(V) and MMAA(III) were much less abundant (<0.1% of initial arsenate) in the arsenic-high media or undetectable in the arsenic-low media. Several researchers have reported organoarsenicals other than methylated forms in natural waters (Howard and Comber, 1989; de Bettencourt and Andreae, 1991, de Bettencourt et al., 1994; Hasegawa et al. 1999). However, no additional organoarsenicals were detected (<0.11 nmol L^{-1}) in any experiment by hydride generation technique after ultraviolet decomposition.

The sum of dissolved arsenite and methylarsenicals was less than the decrement of dissolved arsenate in the culture media. Total arsenic concentrations, which were obtained from unfiltered samples at the end of the three culture experiments (Figs. 1-3), were 98±3% of initial concentrations of arsenate. Therefore, the loss of dissolved arsenic was due to the incorporation of arsenic into the algae. *C. aciculare* incorporated 32-68% of initial arsenate in the arsenic-low media. *C. aciculare* contained 150-350 ppm arsenic in the arsenic-high media and 0.6-11 ppm in the arsenic-low media. Generally, the arsenic content is lower in freshwater algae than marine algae (Phillips, 1990; Maeda, 1994). Although freshwater algae rarely contain more than 1 ppm of arsenic (dry weight), some freshwater algae accumulate arsenic to 10,000-50,000 ppm (Maeda et al., 1985). In our experiments, the content of arsenic in *C. aciculare* increased with the initial concentration of arsenate in the culture media.

3.5 Effect of Phosphate Levels on Methylarsenical Production.

The effect of phosphate on arsenic biotransformation in freshwater algae is shown in Fig. 4. Three strains of freshwater algae (*Closterium aciculare, Aulacoseira granulata, Pediastrum biwae*) were grown in 30 ml test tubes containing 15 ml of modified CT medium with 100 nmol L^{-1} of arsenate and 20 mmol L^{-1} of nitrate until the stationary phase of growth (2-3 weeks after inoculation). Phosphate was initially added to give a range of 1.2-100 μmol L^{-1}. The cell densities of the algae were limited by phosphate concentrations in the phosphate-deficient media (initially 1.2-5.2 μmol L^{-1} for *Closterium* and *Aulacoseira* , and 1.2-11 μmol L^{-1} for *Pediastrum*), which decreased to less than 0.5 μmol L^{-1} during the stationary phase. Nitrate levels were steady throughout the experiments. Under the phosphate-deficient conditions, DMAA(III+V) reached 7.6-9.7, 84-98 and 9.9-63 nmol L^{-1} for *Closterium, Aulacoseira* and *Pediastrum*, respectively, and increased with cell abundance of the algae. MMAA(III+V) ranged 1.5 nmol L^{-1} to 2.8 nmol L^{-1} and were found only in the *Aulacoseira* cultures. On the other hand, methylarsenicals decreased with increases in phosphate concentrations, when cells were grown under phosphate-sufficient conditions (11-100 μmol L^{-1} of initial phosphate concentrations for *Closterium* and *Aulacoseira* , and 20-100 μmol L^{-1} for *Pediastrum*). The phosphate-sufficient media contained more than 50-99% of the initial phosphate amounts until the end of the experiments.

The relation between methylarsenical production and phosphate levels in the phosphate-deficient cultures in Fig. 4 coincided with the arsenic speciation change during the growth of *C. aciculare*. Methylation of arsenate by *C. aciculare* was enhanced when the ratio of phosphate to arsenate in the medium decreased at the initial stage of stationary growth (Figs. 1 and 2). These results accord well with previous reports that the uptake of

arsenate is associated with that of phosphate. Several researchers have reported that arsenate is a competitive inhibitor of phosphate uptake by algal cells in phytoplankton cultures of freshwater (Baker et al., 1983; Maeda et al., 1985; Maeda et al., 1988) and seawater (Sanders, 1979; Sanders and Windom, 1980). In natural phytoplankton communities in Lake Biwa, high concentrations of DMAA(V) were frequently observed when the ratio of phosphate/$\sum$As was below 3 under oxic environment (Sohrin et al., 1997a). Arsenate would be converted to methylarsenicals in *C. aciculare* after the decrease of phosphate in the medium and the incorporation of arsenate into *C. aciculare*. Since the production rate for DMAA(V) remained constant during the exponential phase in the 24-hour experiment (Table 2), it is likely that methylarsenicals were excreted as the metabolites rather than photosynthates of *C. aciculare*. Moreover, the DMAA(V) concentration increased continuously during the stationary phase. DMAA(V) may be produced as a consequence of the breakdown of dead cells. The reported distribution of methylarsenicals in natural waters suggests that seasonal changes in DMAA(V) correlate, not with chlorophyll-a, but with water temperature (Hasegawa, 1997; Sohrin et al. 1997a).

There are, however, a few exceptions to the relation between methylarsenical production and phosphate levels in natural waters. Several species of marine algae are capable of discriminating between the uptake of arsenate and phosphate (Andreae and Klumpp, 1979, Morris et al, 1984). The appearance of methylarsenicals in the sea can occur without the dissolved phosphate levels being necessarily low (Froelich et al., 1985; Howard et al., 1995).

3.6 Arsenic Biotransformation

The novelty of this work is in the examination of changes in arsenic speciation associated with the algal metabolism in the freshwater environment. Arsenite appeared in the culture medium during the exponential phase of cell growth in *C. aciculare*, and was oxidized and/or incorporated by *C. aciculare*. Methylarsenicals, mostly comprised of DMAA(V), accumulated during the stationary phase, while the other organoarsenicals were not observed throughout the experiments. It is likely that DMAA(V) is accumulated as a final arsenic metabolite by living algae and/or as a product of the decomposition of dead algal cells. In natural waters, more than one mechanism would be involved in the uptake of arsenic and the production of methylarsenic. However, similar results that arsenate was replaced initially by arsenite and then by DMAA(V) were reported in unaxeous culture studies which were performed in large-volume coastal seawater enclosures containing natural phytoplankton communities (Sanders, 1993).

In addition, our results showed that methylarsenic(III) species can be produced by phytoplankton in freshwater. Methylarsenic(III) species appeared in the culture medium with increase in DMAA(V), and were oxidized immediately following excretion from the organisms (Fig. 1). Organoarsenic synthesis may occur, therefore, when methylarsenic (III) species appear in the culture medium. Such species are considered to be intermediates in the biosynthetic route from arsenate to organoarsenicals (Challenger, 1945). It is conceivable that MMAA(III) and DMAA(III) were released as metabolites from the biosynthetic pathway for methylarsenicals by *C. aciculare* in our experiments. We have only limited information on factors responsible for the proportion of methylarsenic(III) and methylarsenic(V) species. The most likely explanation is that the levels of methylarsenic(III) species depend on both the rates of production by *C. aciculare* and of oxidation in oxic environments. Methylarsenic(III) species are more susceptible to oxidation than arsenite (Cullen, 1966). In particular, DMAA(III) is thermodynamically

unstable in oxic aquatic solutions. The low production rates of DMAA(III) during the light phase may be due to photochemical oxidation (Table 2). The production rates of arsenite and DMAA(V) were not appreciably influenced by light in such a way that those of DMAA(III) were low during light phase. Methylarsenic(III) species can be produced strictly chemically by the reduction of methylarsenic(V) species in porewater under anoxic conditions (Bright et al., 1994). Methylarsenic(III) and methylarsenic(V) species could be released from anoxic micro-aggregates in the photic zone. Much more work is required to establish models of methylation in aquatic environment.

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Figure Captions

Fig. 1. Effect of phytoplankton activity on the chemical form of arsenic in batch culture of *Closterium aciculare* under phosphate-deficient arsenic-high conditions. ○, arsenate; ●, arsenite; □, DMAA(V); △, MMAA(V); ■, DMAA(III); ▲, MMAA(III); ◆, cell density of *C. aciculare* ; ▼, phosphate; ◇, nitrate.

Fig. 2. Effect of phytoplankton activity on the chemical form of arsenic in batch culture of *Closterium aciculare* under phosphate-deficient arsenic-low conditions. ○, arsenate; ●, arsenite; □, DMAA(V); ◆, cell density of *C. aciculare* ; ▼, phosphate; ◇, nitrate.

Fig. 3. Effect of phytoplankton activity on the chemical form of arsenic in batch culture of *Closterium aciculare* under nutrient-deficient arsenic-low conditions. ○, arsenate; ●, arsenite; □, DMAA(V); ■, DMAA(III); ◆, cell density of *C. aciculare* ; ▼, phosphate; ◇, nitrate.

Fig. 4. Effect of phosphate levels on the arsenic metabolism of freshwater algae. Arsenic species were determined at the beginning of the stationary phase. (a)*Closterium aciculare,* (b)*Aulacoseira granulata,* (c)*Pediastrum biwae.* □, DMAA(III+V); △, MMAA(III+V); ◆, dry weight.

Table 1 Initial Concentrations of Arsenate and Nutrients in the Culture Media.

Type	Arsenate (mol L^{-1})	Phosphate (mol L^{-1})	Nitrate (mol L^{-1})
Phosphate-deficient arsenic-high medium	1.2×10^{-5}	1.9×10^{-5}	2.2×10^{-2}
Phosphate-deficient arsenic-low medium	1.0×10^{-8}	1.1×10^{-5}	1.9×10^{-2}
Nutrient-deficient arsenic-low medium	1.0×10^{-8}	1.1×10^{-5}	2.1×10^{-5}

Table 2 Average Production Rates for Arsenicals and Uptake Rates for Phosphate in the Period of Growth of *Closterium aciculare* from 3.7×10^3 to 6.2×10^3 cells mL^{-1}.

	Light Phase	Dark Phase
Production rate		
Cell Density (cells mL^{-1} h^{-1})	190±20	23±5
Arsenite (nmol L^{-1} h^{-1})	8.85±0.74	9.49±2.06
DMAA(V) (nmol L^{-1} h^{-1})	0.45±0.05	0.39±0.03
DMAA(III) (nmol L^{-1} h^{-1})	0.03±0.03	0.19±0.04
Uptake rate		
Phosphate (nmol L^{-1} h^{-1})	74.3±5.1	12.0±0.6

Table 3 Arsenic Distribution in the *C. aciculare* Cultures during the Stationary Phase.

Type	Arsenate (%)	Arsenite (%)	DMAA(V) (%)	MMAA(V) (%)	DMAA(III) (%)	MMAA(III) (%)	Incorporated arsenic (%)	Incorporated arsenic (ppm)
Phosphate-deficient arsenic-high medium	70-85	0.3-1.0	8.2-16	<0.1	<0.1	<0.1	<0.1	150-350
Phosphate-deficient arsenic-low medium	1.0-12	<0.1	24-43	nd[a]	nd[a]	nd[a]	49-68	0.6-1.4
Nutrient-deficient arsenic-low medium	51-62	20-27	4.3-6.1	nd[a]	0.5-1.7	nd[a]	32-43	5.9-11

[a]Not detected (nd).

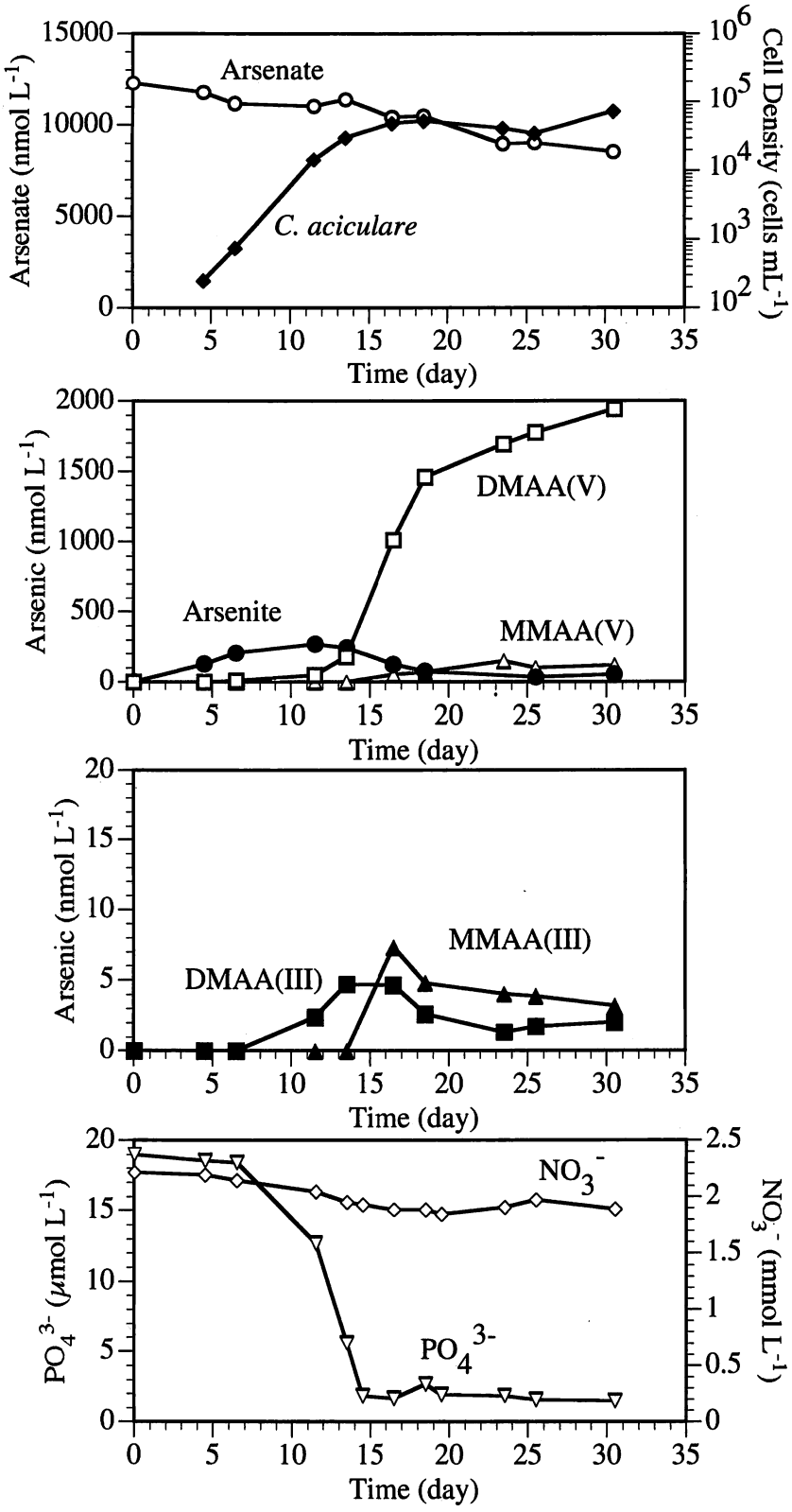
Arsenate
C. aciculare
Arsenate (nmol L-1)
Cell Density (cells mL-1)
Time (day)
DMAA(V)
Arsenite
MMAA(V)
Arsenic (nmol L-1)
Time (day)
MMAA(III)
DMAA(III)
Arsenic (nmol L-1)
Time (day)
NO3-
PO43-
PO43- (μmol L-1)
NO3- (mmol L-1)
Time (day)

Fig. 1

Table 3 Arsenic Distribution in the *C. aciculare* Cultures during the Stationary Phase.

Type	Arsenate (%)	Arsenite (%)	DMAA(V) (%)	MMAA(V) (%)	DMAA(III) (%)	MMAA(III) (%)	Incorporated arsenic (%)	Incorporated arsenic (ppm)
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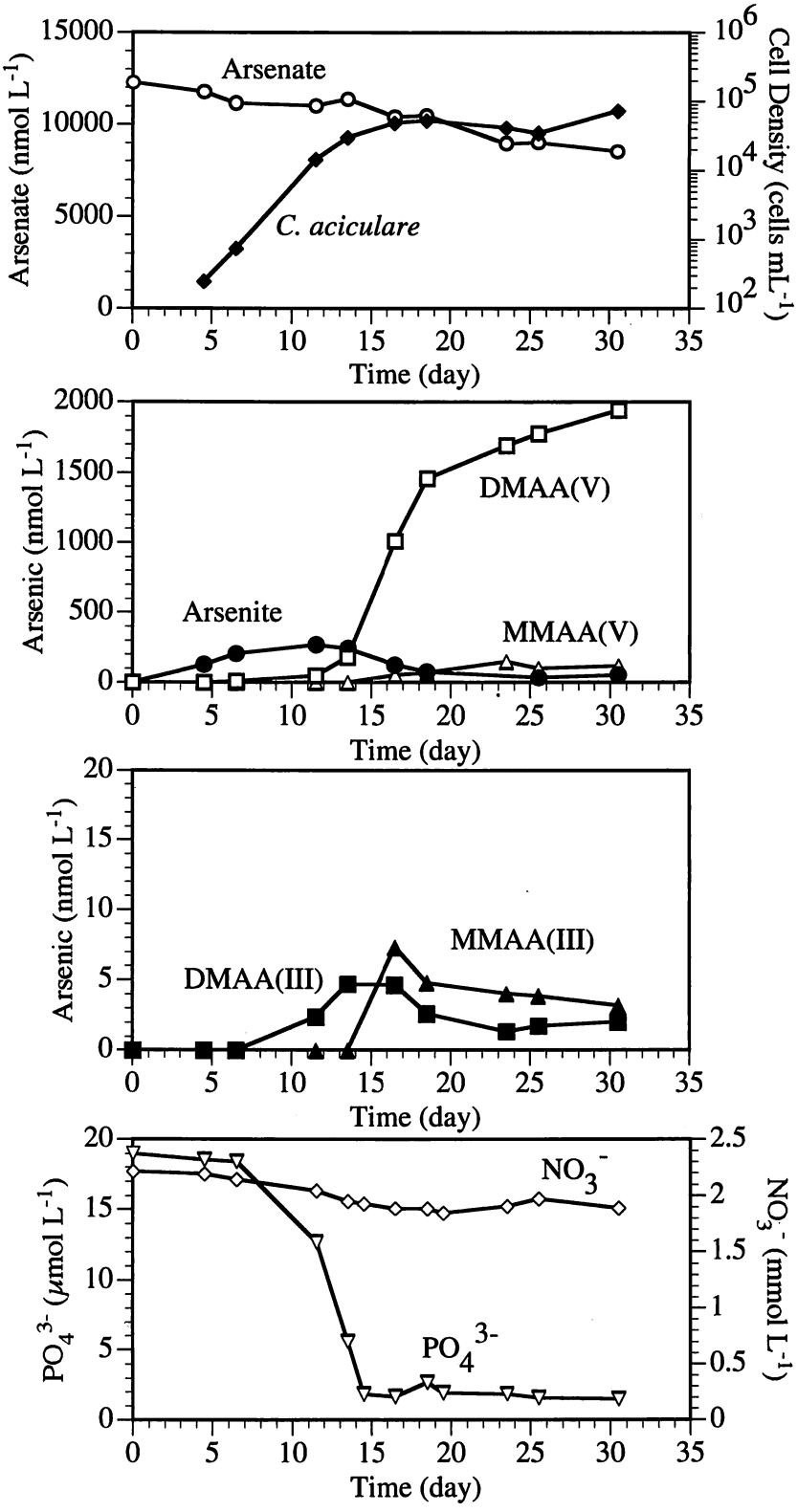
Arsenate
C. aciculare
Arsenate (nmol L-1)
Cell Density (cells mL-1)
Time (day)
DMAA(V)
Arsenite
MMAA(V)
Arsenic (nmol L-1)
MMAA(III)
DMAA(III)
NO3-
PO43-
PO43- (μmol L-1)
NO3- (mmol L-1)

Fig. 1

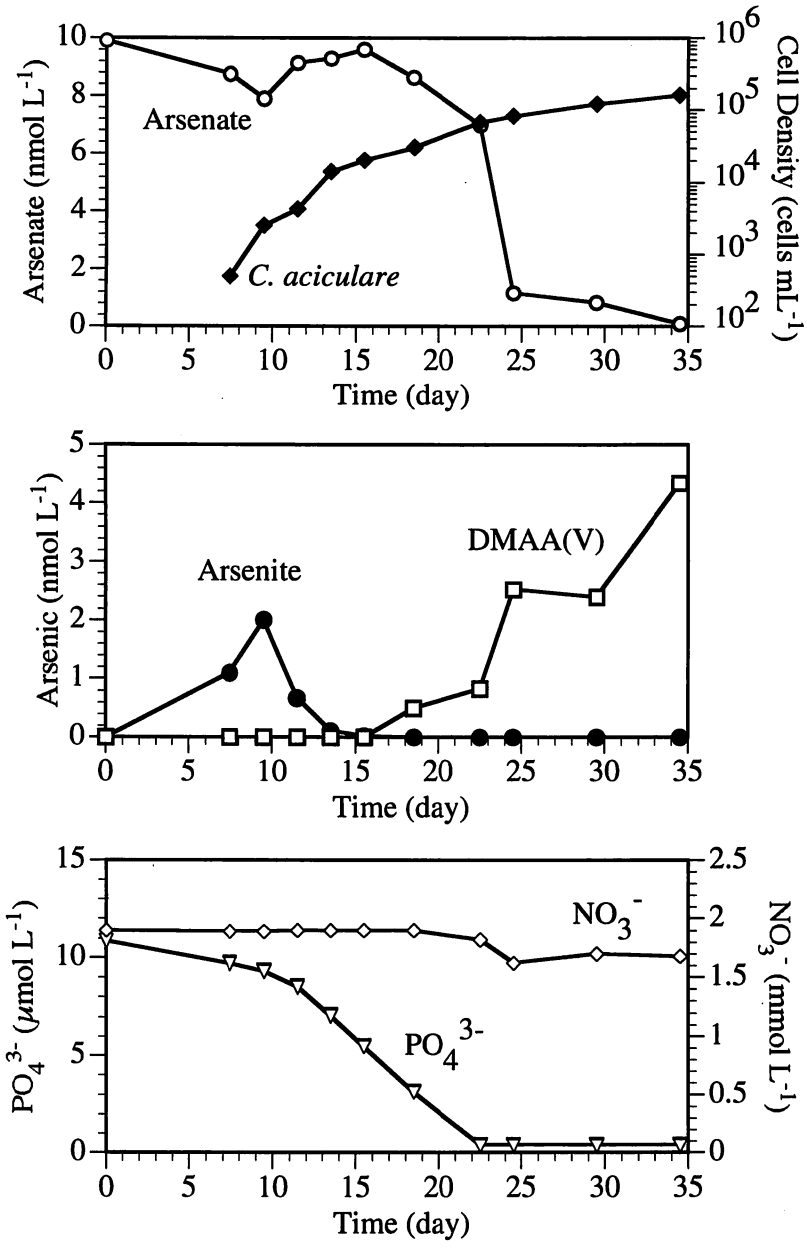
Arsenate
C. aciculare
Arsenate (nmol L-1)
Cell Density (cells mL-1)
Time (day)
Arsenite
DMAA(V)
Arsenic (nmol L-1)
Time (day)
NO3-
PO4 3-
PO4 3- (μmol L-1)
NO3- (mmol L-1)
Time (day)

Fig. 2

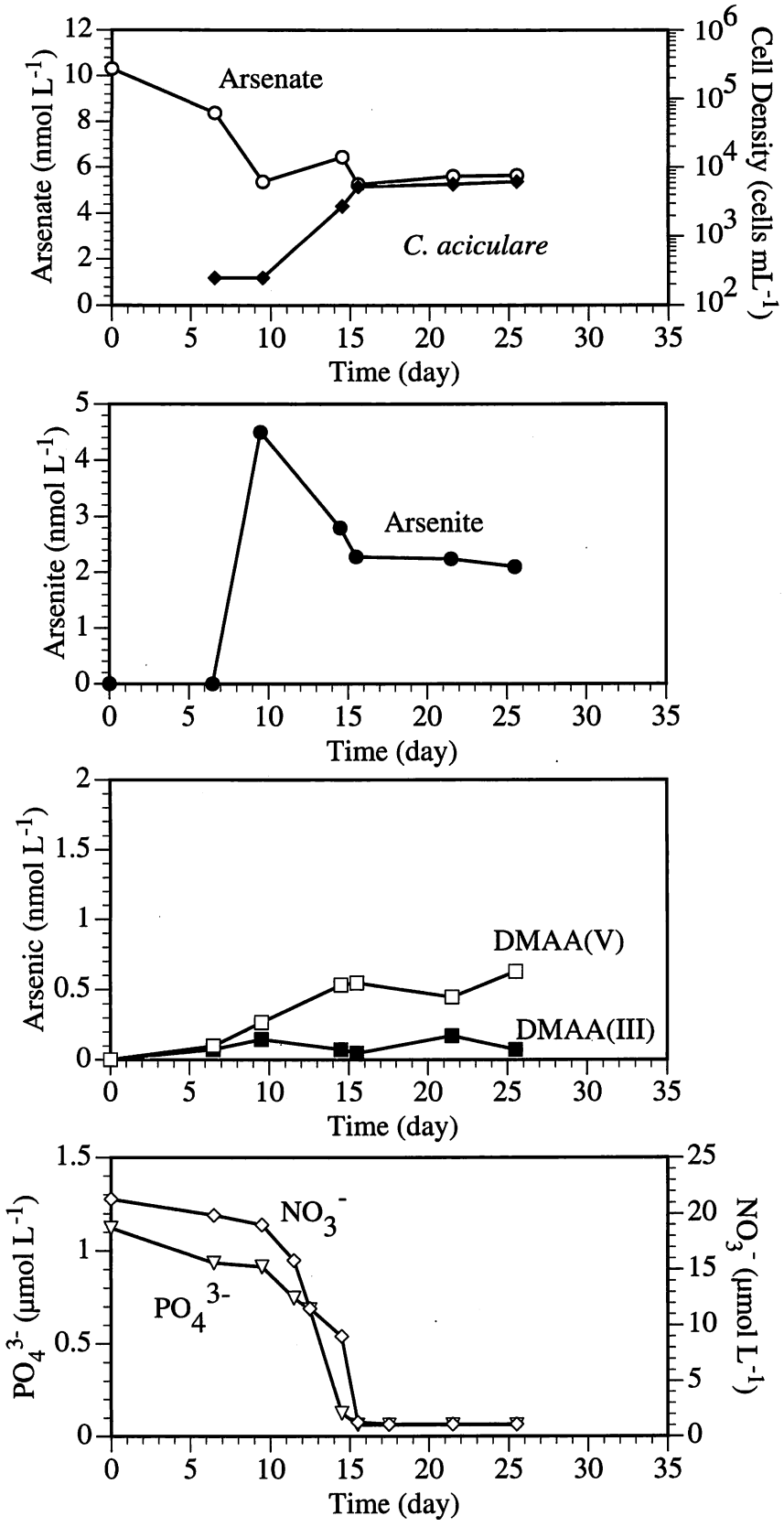
Arsenate
C. aciculare
Arsenate (nmol L-1)
Cell Density (cells mL-1)
Time (day)
Arsenite
Arsenite (nmol L-1)
Time (day)
DMAA(V)
DMAA(III)
Arsenic (nmol L-1)
Time (day)
NO3-
PO43-
PO43- (µmol L-1)
NO3- (µmol L-1)
Time (day)

Fig. 3