Influence of aggregated particles on biodegradation activities for dimethylarsinic acid (DMA) in Lake Kahokugata

Title:

Influence of aggregated particles on biodegradation activities for dimethylarsinic acid (DMA) in Lake Kahokugata

Running head:

Biodegradation of dimethylarsinic acid by aggregates

Authors:

Teruya Maki[1]*, Wakana Hirota[1], Hiroyuki Motojima[1], Hiroshi Hasegawa[1] and Mohammad Azizur Rahman[1]

Affiliation of all authors:

1 College of Science and Engineering, Kanazawa University, Kakuma-machi, Kanazawa-shi, 9201192, Japan.

*Corresponding author:

Tel: +81-(0) 76-234-4793, Fax: +81-(0) 76-234-4800

E-mail: makiteru@t.kanazawa-u.ac.jp

Abstract

Aquatic arsenic cycles mainly depend on microbial activities that change the arsenic chemical forms and influence human health and organism activities. The microbial aggregates degrading organic matter are significantly related to the turnover between inorganic arsenic and organoarsenic compounds. We investigated the effects of microbial aggregates on organoarsenic mineralization in Lake Kahokugata using lake water samples spiked with dimethylarsinic acid (DMA). The lake water samples converted 1 μmol L^{-1} of DMA to inorganic arsenic for 28 days only under anaerobic and dark conditions in the presence of microbial activities. During the DMA mineralization process, organic aggregates >5.0 μm with bacterial colonization increased the densities. When the organic aggregates >5.0 μm were eliminated from the lake water samples using filters, the degradation activities were reduced. DMA in the lake water would be mineralized by the microbial aggregates under anaerobic and dark conditions. Moreover, DMA amendment enhanced the degradation activities in the lake water samples, which mineralized 50 μmol L^{-1} of DMA. The DMA-amended aggregates >5.0 μm completely degraded 1 μmol L^{-1} of DMA with a shorter incubation time of 7 days. The supplement of KNO_3 and $NaHCO_3$ to lake water samples also shortened the DMA degradation period. Presumably, the bacterial aggregates involved in the chemical heterotrophic process would contribute to the DMA-biodegradation process in Lake Kahokugata, which is induced by the DMA amendment.

Key words; arsenic cycle, organoarsenic compounds, biodegradation, aggregate

Introduction

The arsenic species in aquatic ecosystems changed their chemical forms mainly dependently on the microbial activities that metabolize the arsenic species, and some arsenic chemical forms are known to endanger human health and organism activities at high concentrations (Cullen and Reimer, 1989, Neumann et al., 2010). Among the variety of arsenic species, arsenate, arsenite, and methyl arsenic compounds dominate in both fresh water and seawater environments (Oremland and Stolz, 2003). Microorganisms, such as phytoplankton (microalgae) and bacteria, methylate arsenate into monomethylarsonic acid ($CH_3AsO(OH)_2$; MA(V)) and dimethylarsinic acid ($(CH_3)_2AsO(OH)$; DMA(V)) and subsequently convert the methyl arsenic compounds to more complex organoarsenic compounds, such as arsenobetain and arsenosugar (Howard and Comber, 1989). However, a significant positive correlation between the *in situ* amounts of chlorophyll a (the biomass of microalgae) and those of organoarsenic compounds has not been found, because the environmental degradation of organoarsenic compounds by bacteria had led to this inconsistency (Sohrin et al., 1997).

The dominant chemical forms of arsenic compounds in a number of lakes and estuaries have been reported to change seasonally by the degradation and production of organoarsenic compounds (Anderson and Bruland, 1991, Maki et al., 2009). A few reports have described that bacterial consorcia in marine environments could degrade the amended organoarsenic compounds (Sanders, 1979, Kaise et al., 1985) and that bacterial population associated with crabs (Khokiattiwong et al., 2001) and mussels (Jenkins et al., 2003) also had the degradation activities for organoarsenic compounds.

66 The several species of bacterial isolates degrading organoarsenic compounds were
67 obtained from activated sludge (Quinn and McMullan, 1995) and natural environments
68 (Lehr et al., 2003, Maki et al., 2006). The degradation process of organoarsenic
69 compounds is worthy of study for understanding the ecological dynamics and
70 distribution of arsenic compounds in aquatic environments.

71 In aquatic environments, organic aggregates colonized by heterotrophic
72 microorganisms are important components in the turnover of both organic and inorganic
73 matter (Grossart and Ploug, 2000). The bacteria population attached to organic
74 aggregates has been reported to exhibit higher metabolic activities than free-living
75 bacterial population in the surrounding waters (Caron et al., 1982, Ghiglione et al.,
76 2007). Organic aggregates and their surrounding environments are the hot spots of the
77 microbial process, during which the degradation activities of organic matter are
78 expanded (Crump et al., 1999). In the organic aggregates where concentration gradients
79 of oxygen develop, the organic-degradation activities of anaerobic bacteria, such as
80 denitrifying bacteria and methane-synthetic bacteria, are high (e.g. Oremland 1979).
81 Our previous study demonstrated that the DMA biodegradation in lake water was
82 activated only under anaerobic and dark condition, indicating the possibility that the
83 organic aggregates provide the hot spots for the DMA-degrading microorganisms and
84 support the DMA-biodegradation (Maki et al. 2009). However, the actual
85 biodegradation of DMA by organic aggregates with bacterial colonization is still
86 unknown.

87 In this study, the influence of aggregates on the DMA-biodegradation process
88 were estimated in detail using lake water samples that had been collected from a single

lake, Lake Kahokugata, and spiked with DMA. We also compared the DMA-degradation activities between DMA-amended and unamened lake water to evaluate the activation of DMA degradation by the amendment. The microbial consocia amended with some chemical substrates were reported to elevate the biodegradation for the chemical substrates (e.g. Eilers et al. 2000). Moreover, for elucidating the metabolic characteristics of bacterial consocia relating with DMA degradation, the chemical substrates that activate the anaerobic bacteria, such as denitrifying bacteria and methane-synthetic bacteria, were added to lake water samples and the induction of DMA-biodegradation activities was evaluated. DMA was selected as a representative organoarsenic compound that is widely distributed in freshwater (Sohrin et al., 1997).

Materials and Methods

Sampling at Lake Kahokugata

A water sample at the depth of 1 m was collected in polycarbonate bottles from Lake Kahokugata in Ishikawa Prefecture, Japan. Lake Kahokugata is eutrophic and has experienced wastewater inflow from cities and croplands. Lake Kahokugata is shallow with a depth of less than 2 m and the water was frequently mixed throughout the four seasons. The oxygen levels in the lake water sample ranged from 2.0 mg L^{-1} to 8.3 mg L^{-1} during the investigation period. When the water transparency was measured using a standard 25 cm black and white Secchi disk, the disk depth was approximately 1.0 m from the water surface at the sampling dates, indicating that the sun irradiation hardly reached a depth below 1 m. After at least 2 hours of sampling, the surface water samples

of Lake Kahokugata in polycarbonate bottles were used for several culture experiments, in which we estimated the DMA-biodegradation activities of lake water under several experimental treatments and the microbial dynamics during the DMA-degradation process. The concentrations of inorganic arsenic and organoarsenic compounds in the lake water without the addition of DMA were stable below 20 nmol L^{-1} during the entire experiment.

Influences of the microbial activities on DMA biodegradation in lake water

Lake water samples collected in polycarbonate bottles from Lake Kahokugata on 29 August 2008 were used to investigate the DMA-biodegradation activities in lake water samples incubated under anaerobic and dark conditions. Fifteen polycarbonate bottles (500 mL) were filled up with lake water and transferred to our laboratory. Within 2 hours of sampling, 500 µL of a 1 mmol L^{-1} DMA (Nacalai Tesque, Kyoto, Japan) solution was added into 3 bottles, and the final concentration of DMA was adjusted to 1 µmol L^{-1}. The bottles were incubated under anaerobic and dark conditions. To produce the anaerobic conditions, the air phases in the bottles were kept at the lowest possible level, and the lake water samples were purged with nitrogen (100 mL min^{-1}) for 0.5 hours. The bottles were incubated in a temperature-controlled room (20 ºC) under dark conditions by covering the bottles with aluminum foil. The anaerobic and dark condition of incubation was optimal for DMA-degradation in lake water (Maki et al., 2009).

The remaining 12 bottles were used for the experiment that the microbial activities in the lake water samples were eliminated using four treatments: the lake

135 water sample was autoclaved at 120 ºC for 20 minutes; an antibiotic mixture was added
136 to each sample of lake water at a final concentration of 10 mg L^{-1}; sodium azide was
137 added to each sample of lake water at a final concentration of 10 mg L^{-1}; and the lake
138 water was filtrated through a 0.02 μm pore-size filter polycarbonate filter (Whatman,
139 Tokyo, Japan). Each treatment was performed using triplicate bottles. The bottles of the
140 lake water samples treated by each method and spiked with 1 μmol L^{-1} DMA were
141 incubated at 20 ºC under anaerobic and dark conditions.
142 At 0, 14, 21, 28 and 56 days of the incubation, portions (10 mL) of the lake
143 water samples were collected, and the concentrations of arsenic species were
144 determined using a cold-trap hydride-generation atomic-absorption (HG-AA) speciation
145 procedure. After sampling of subsamples, the air space volumes in the bottles were
146 exchanged by nitrogen.
147
148 **Effects of aggregate sizes in lake water on DMA biodegradation**
149 The lake water samples, which were collected from Lake Kahokugata on 22
150 May 2008, were filtrated through polycarbonate filters with four pore sizes of 10, 5.0,
151 1.2, and 0.22 μm (Millipore, Tokyo, Japan). Within 2 hours of sampling, each filtrate
152 was placed in polycarbonate bottles (500 mL) and spiked with DMA added at a final
153 concentration of 1 μmol L^{-1}. Triplicate bottles were prepared for each sample of filtrate,
154 and incubated at a temperature of 20 ºC under anaerobic and dark conditions as
155 described. At every 7 or 14 days of the incubation period (56 days), portions (10 mL) of
156 the lake water samples were collected to measure the concentrations of arsenic species.
157

Induction of DMA biodegradation in the DMA-amended lake water samples

For investigating the activation of DMA degradation by the DMA amendment, the lake water samples collected on 22 May 2008 were spiked with 1 μmol L^{-1} of DMA and amended at 20 ºC under anaerobic and dark conditions for 28 days (DMA-amended lake water sample). DMA in the DMA-amended lake water samples was completely converted to inorganic arsenic within 28 days of incubation. The 500 mL DMA-amended lake water samples were sequentially filtrated through a series of polycarbonate filters with pore sizes of 5.0 and 1.2 μm (Millipore, Tokyo, Japan). The aggregates on the 5.0 and 1.2 μm filters were added to 500 mL lake water samples that were collected on 9 June 2008 and filtrated through a 0.02 μm pore-size polycarbonate filter. The filtrate with the 1.2 μm filters was also used for the following experiments as the lake water sample including <1.2 μm aggregates. Finally, the lake water samples including the aggregates separated into three fractions: > 5.0 μm, 5.0 μm to 1.2 μm, and <1.2 μm were prepared. One μmol L^{-1} of DMA was spiked to the lake water samples including each fraction of aggregates and unfiltrated DMA-amended lake water sample (500 mL). The water samples were incubated at 20 ºC under anaerobic and dark conditions. At every several days of incubation, the concentrations of arsenic species in portions (10 mL) of the lake water samples were measured.

Next, to evaluate the concentration capacities of DMA-degradation in DMA-amended lake water, 5 polycarbonate bottles (500 mL) were filled up with lake water collected from Lake Kahokugata on 22 May 2008. Four bottles were amended with 1 μmol L^{-1} of DMA for 28 days (DMA-amended lake water sample), and the other bottle was incubated without DMA amendment for 28 days (unamended lake water

sample). The 4 bottles of DMA-amended lake water samples were then spiked with DMA at the final concentrations of 1, 5, 10, and 50 μmol L^{-1}, and the bottle of unamended lake water samples was also spiked with 10 μmol L^{-1} DMA. These bottles were continuously incubated at 20 ºC under anaerobic and dark conditions. At every 7 days after the second DMA spike, portions (10 mL) of the lake water samples were collected to determine the concentrations of arsenic species.

Effects of chemical substrates on DMA biodegradation in lake water

The polycarbonate bottles (500 mL) were filled up with lake water samples collected from Lake Kahokugata on 11 September 2009. The lake water samples were supplemented with one of 10 kinds of chemical substrates; $NaHCO_3$ (final concentration: 1.0, 4.0, and 10 g L^{-1}), KNO_3 (1.0, 4.0, and 10 g L^{-1}), Na_2SO_4 (4.0 g L^{-1}), $MgSO_4$ (4.0 g L^{-1}), $CuSO_4 \cdot 5H_2O$ (4.0 g L^{-1}), $FeSO_4 \cdot 7H_2O$ (4.0 g L^{-1}), CH_3COONa (4.0 g L^{-1}), L-g1ucose (1.0 g L^{-1}), yeast extract (1.0 g L^{-1}), or L-cystin (1.0 g L^{-1}) (Nacalai Tesque, Kyoto, Japan). The amendment using $NaHCO_3$ and KNO_3 increase the activities of methan-synthetic and nitrogen-reducing bacterial consortia in lake water samples. The additon of Na_2SO_4, $MgSO_4$, $CuSO_4 \cdot 5H_2O$, and $FeSO_4 \cdot 7H_2O$ activate the sulfate-reducing bacterial consortia. The addition of CH_3COONa, L-g1ucose, yeast extract, and L-cystin increase the cometabolic activities of several bacterial communities. Lake water samples without chemical-substrate addition were also prepared as controls. DMA was added to the lake water samples at a final concentration of 1 μmol L^{-1}, and each experiment was performed using triplicate bottles. After 21 days of incubation at 20 ºC under anaerobic and dark conditions, the concentrations of

arsenic species in the lake water samples were determined.

Measurements of the arsenic compound concentration

The cold-trap HG-AA speciation procedure was employed as the protocol previously reported (Braman and Foreback, 1973). The water subsamples were filtrated through a 0.45 μm cellulose ester filter (Millipore, Tokyo, Japan) and acidified and reacted with 10% w/v sodium tetrahydroborate. The produced arsines were swept by a flow of nitrogen into a cold-trap column cooled by liquid nitrogen. After the column was gently warmed by electrical heating, the arsines released from the column were loaded into a quartz-T tube held at ca. 900 ˚C in a flame in order to inorganic arsenic (arsenate), MMA and DMA, and quantified using an atomic absorption spectrometer Z-8100 (Hitachi, Chiba, Japan). In this experiment, the accuracy of the measured values was considered as 10 nmol L^{-1}.

Observation of particles in lake water samples using epifluorescence microscopy

The 500 mL lake water samples collected in 3 polycarbonate bottles from Lake Kahokugata on 22 May 2008 were spiked with DMA at a final concentration of 1 μmol L^{-1}. For 56 days of incubation at 20 ºC under anaerobic and dark conditions, a 1-mL water subsample was collected from each bottle every week. The water subsamples were fixed with a glutaraldehyde solution at a final concentration of 1%. The samples were stained with DAPI (4',6-diamino-2-phenylindole) at a final concentration of 0.5 μg mL^{-1} for 15 min and filtrated through a 0.22 μm pore-size polycarbonate filter (Millipore, Tokyo, Japan) stained with Sudan Black (Russell et al., 1974). After the

filter was placed on a slide on top of a drop of low-fluorescence immersion oil, a drop of oil was added and then covered with a cover slide. Slides were examined using an epifluorescence microscope (Olympus, Tokyo, Japan) under UV excitation. Under the microscopic observation, the free-living bacterial particles in lake water samples were observed as coccoid-like or bacillus-like particles with white-blue fluorescence with a diameter of below 1.0 μm. Furthermore, the particles with yellow fluorescence ranging from 1.0 μm to about 500 μm diameters were also observed. Among the yellow fluorescing particles, some of them were colonized with bacterial particles, and the others were not attached with the bacterial particles. A filter transect was scanned and the particles on the filter transect, which could be discriminated into free-living bacterial particles, yellow fluorescing particles without bacterial-particle attachment, and yellow fluorescing particles with bacterial colonization in two size categories (<5.0 μm and >5.0 μm), were enumerated. Each particle count was performed on 10 microscopic fields randomly selected.

Results

Influence of the microbial activities on DMA biodegradation in lake water

When the lake water samples were spiked with DMA at a final concentration of approximately 1 μmol L^{-1} and incubated at 20 ˚C under anaerobic and dark conditions, the concentration of DMA at the onset of the experiment decreased from 916 nmol L^{-1} (average) to the detection limit (avg.) during the first 28 days of incubation (Table 1). In accordance with the decrease of DMA, the concentration of inorganic arsenic, which

250 is considered to be the resultant product from DMA degradation, increased to 912 nmol
251 L^{-1} at the 28th day and fluctuated the concentration of 900 nmol L^{-1} until 56 days of
252 incubation. In contrast, when the microbial activities in the lake water samples were
253 eliminated, the concentrations of DMA maintained for 56 days of incubation (Table 1).
254 In the lake water samples that microbial activities were removed, no significant
255 difference among incubation days were determined by the analysis of one-way ANOVA
256 test. These results indicated that this DMA degradation occurred as a result of a biotic
257 (microbiological) process under anaerobic and dark conditions.
258
259 **Effects of aggregate size in lake water on DMA biodegradation**
260 In this lake water samples spiked with DMA, the yellow fluorescing particles
261 without bacterial particles decreased the densities from 3.2×10^4 to 7.2×10^3 particles
262 mL^{-1} during the first 14 days of incubation and maintained low densities until 56 days
263 (Fig. 1). The densities of yellow fluorescing particles <5.0 μm with bacterial
264 colonization rapidly increased from 6.4×10^3 to 3.3×10^4 particles mL^{-1} during the first
265 7 days and decreased to 7.2×10^3 particles mL^{-1} on the 21st day, maintaining the order
266 of 10^3 particles mL^{-1} until the 56th day. The densities of yellow fluorescing particles
267 >5.0 μm with bacterial colonization gradually increased from 1.5×10^3 to 2.9×10^4
268 particles mL^{-1} for 28 days, and fluctuated over densities of 1.6×10^4 particles mL^{-1} until
269 the 56th day. The free-living bacterial cells maintained concentrations ranging from 10^4
270 to 10^5 cells mL^{-1} during the experimental period. The total densities of bacterial particles
271 colonized on yellow fluorescing particles >5.0 μm were ranged from 2.0×10^4 to $3.2 \times$
272 10^5 particles mL^{-1} for 28 days (data not shown), suggesting that the total biomasses of

free-living and aggregate bacteria in the lake water were mostly equal at order levels.

When the filtrated and unfiltrated lake water samples were spiked with approximately 1 μmol L^{-1} of DMA, the lake water samples filtrated through a 10 μm pore-size filter degraded 90 % of DMA and accumulated inorganic arsenic at a concentration of 860 nmol L^{-1} within 28 days, indicating a similar rate of DMA degradation to the unfiltrated lake water samples (Fig. 2a, b). In contrast, DMA spiked to filtrate samples through 5.0 μm and 1.2 μm pore-size filters significantly mineralized DMA for longer incubation times (42 to more than 56 days, respectively) (Fig. 2c, d), suggesting that the DMA-degradation activities decreased in the lake water samples filtrated through pore-size filters of less than 5.0 μm. In the lake water samples filtrated through the 0.22 μm pore-size filter that could eliminate bacterial cells, DMA degradation was diminished (Fig. 2e).

Induction of DMA biodegradation in the DMA-amended lake water samples

After the lake water samples were incubated for 28 days with DMA, DMA was added to the DMA-amended lake water samples at a concentration of 1 μmol L^{-1} again. The DMA spiked into the DMA-amended lake water samples decreased rapidly, and the inorganic arsenic increased the concentrations from 964 to 1715 nmol L^{-1} within 7 days from the second spiked time of DMA (Table 2). In the unamended lake water sample, DMA spiked was converted to inorganic arsenic for long incubation times of 21 days from the second spiked time of DMA. When several sizes of aggregates collected from the DMA-amended lake water samples were added to the lake water samples filtrated through a 0.02 μm filter, the 1 μmol L^{-1} of DMA in the samples including aggregates

296 >5.0 μm was completely converted to inorganic arsenic in 7 days (Table 2). The lake
297 water samples including aggregates 1.2-5.0 μm or <1.2 μm completely mineralized the
298 DMA for longer incubation times of 21 days and 28 days, respectively (Table 2).
299 Consequently, aggregates >5.0 μm in the DMA-amended lake water samples kept the
300 high activities of DMA biodegradation in lake water.

301 Furthermore, when the DMA-amended lake water samples were spiked with
302 DMA at four concentrations of 1, 5, 10, and 50 μmol L^{-1}, the DMA was completely
303 degraded within 7, 14, 28, and 200 days of incubation, respectively (Fig. 3a, b, c, d). In
304 the unamended lake water samples including 10 μmol L^{-1} of DMA, up to 4 μmol L^{-1} of
305 DMA could be degraded to inorganic arsenic for 56 days, and the DMA degradation
306 ceased on the 200th day (Fig. 3e). These results mean that the DMA-amended lake
307 water samples possessed higher activities of DMA degradation than the unamended lake
308 water samples.

309

310 **Effects of chemical substrates on DMA biodegradation in lake water**

311 When the 10 kinds of chemical substrates were added to lake water samples
312 spiked with DMA, only the lake water samples including KNO_3 and $NaHCO_3$
313 significantly mineralized 1 μmol L^{-1} of DMA on the 21st day of incubation (Table 3). In
314 the lake water samples supplemented with 4.0 g L^{-1} of KNO_3 and $NaHCO_3$, DMA was
315 remarkably decreased, and inorganic arsenic increased to over 900 nmol L^{-1} on the 21st
316 day of incubation. The lake water samples including 4.0 g L^{-1} of KNO_3 and $NaHCO_3$
317 were different as compared the lake water samples without chemical-substrate addition
318 and including the other 8 chemical substrates at the $P<0.01$ (One-way ANOVA test). In

319 contrast, the lake water samples with 1.0 and 10 g L^{-1} KNO_3 and $NaHCO_3$ showed the
320 wide range of DMA-degradation rates ranging from 25 % to 100 % on the 21st day.
321 These results suggested that the addition of KNO_3 and $NaHCO_3$ at proper
322 concentrations, such as 4.0 g L^{-1}, enhanced the initiation of DMA degradation in the
323 lake water samples. During the experimental period of each chemical supplement, the
324 pH in lake water samples ranged from 7.5 to 8.0, suggesting that the effects of pH on
325 the degradation could be neglected (data not shown).
326
327 **Discussion**
328
329 Some microalgae in lake water and coastal seawater incorporate and accumulate
330 inorganic arsenics instead of phosphorus and synthesize methyl-arsenic compounds for
331 detoxification (Andreae, 1979, Hasegawa et al., 2001). However, a positive correlation
332 between the *in situ* amounts of microalgal biomass and methyl-arsenic compounds was
333 hardly observed in aquatic environments, and the mineralization of methyl-arsenic
334 compounds is considered to be responsible for cause this inconsistency (Anderson and
335 Bruland, 1991, Sohrin et al., 1997). Sanders (1979) demonstrated that natural freshwater
336 samples with DMA amendment exhibited DMA-degradation activities. Although
337 organoarsenic compound in the terrestrial environments are volatilized as well as were
338 mineralized to inorganic arsenic, the rates of DMA degradation was much greater than
339 those of arsenic evolution from the terrestrial environments to atmosphere (Gao and
340 Burau, 1997). In this study, when DMA was spiked into natural lake water samples
341 collected from Lake Kahokugata, the concentration of inorganic arsenic increased in

342 accordance with the decrease of DMA under anaerobic and dark conditions (Table 1).
343 The increase concentrations of inorganic arsenic after DMA disappearance were equal
344 approximately to the decrease concentrations of DMA. Therefore, the more than 95 %
345 of DMA spiked to the lake water samples would be degraded and converted to inorganic
346 arsenic. Furthermore, when microbial activities were eliminated from the lake water
347 samples, the degradation of DMA and the accumulation of inorganic arsenic diminished
348 (Table 1). In estuaries, biological demethylation has been reported to be the dominant
349 process for the generation of inorganic arsenic from organoarsenic compounds
350 (Anderson and Bruland, 1991). In Lake Kahokugata, DMA degradation is thought to
351 constantly occur as a result of a biotic (microbiological) process.

352 The epifluorescence microscopy observation using the DAPI staining technique
353 revealed that the number of yellow fluorescing particles >5.0 μm with bacterial
354 colonization increased during the DMA-degradation process (Fig. 1). Moreover, the
355 lake water samples filtrated through >5.0 μm pore-size filters showed similar rates of
356 DMA biodegradation to unfiltrated lake water samples, while the lake water samples
357 filtrated through <5.0 μm pore-size polycarbonate filters completely degraded DMA for
358 longer incubation times (more than 42 days) (Fig. 2). The bacteria-attached aggregates,
359 which are stained with DAPI as yellow fluorescing particles, have important
360 implications for DMA biodegradation in lake water. DAPI yellow fluorescing particles
361 have been demonstrated to resemble detritus originated from planktonic materials on
362 copepod fecal pellets (Mostajir et al., 1995). Some microorganisms in lake water and
363 coastal seawater, such as fungi, bacteria, and phytoplankton, are known to accumulate
364 inorganic arsenics and produce organoarsenic compounds (Andreae, 1979, Hasegawa et

al., 2001). Therefore, in natural environments, the microbial communities on/within the organic aggregates are thought to degrade the organoarsenic compounds, such as DMA, which are synthesized by microorganisms and included in the aggregates.

During the 28 days of the DMA-biodegradation period, the number of DAPI yellow fluorescing particles >5.0 μm with bacterial colonization increased (Fig. 1), suggesting that the bacteria form their colonies on organic aggregates >5.0 μm during the incubation period. Furthermore, the aggregates >5.0 μm obtained from the DMA-amended lake water samples also showed higher rates of DMA degradation than those of the unamended lake water samples (Table 2). Since the total cell densities of free-living bacteria and aggregate bacteria were mostly equal, the aggregate bacterial cells would have higher degradation activities than the free-living bacteria cells. The microbial degradation rates of organic matter on aggregates have been reported to be much higher than those estimated by the biomass production of aggregates (Ghiglione et al., 2007). Several studies found that the bacteria associated with aggregates exhibit significantly higher potential ectoenzymatic hydrolysis rates per bacterial cell, implying that organic matter is hydrolyzed faster on aggregates than in the surrounding water (Unanue et al., 1998). The formation of microbial colonies associated with the aggregates >5.0 μm would be an essential for the initiation of DMA degradation in lake water.

In the DMA-amended lake water samples and the lake water samples containing DMA-amended aggregates >5.0 μm, 1 μmol L^{-1} DMA was rapidly converted to inorganic arsenic in 7 days (Table 2). On the contrary, DMA in the unamended lake water sample was completely degraded for longer incubation times of 21 days.

388 Furthermore, the DMA-amended lake water samples completely mineralized at least 50
389 μmol L^{-1} of DMA, whereas the unamended lake water sample could mineralize a
390 maximum of 4 μmol L^{-1} of DMA (Fig. 3). These results suggested that DMA
391 amendment activated the DMA-biodegradation activities of organic aggregates in the
392 lake water. Bacteria attached onto 5- to 14-day-old aggregates exhibited higher
393 respiration rates than those attached onto <5-day-old aggregates (Grossart and Ploug,
394 2000). In addition, enrichment of the microorganisms with organic compounds has been
395 reported to influence the microbial species compositions and their metabolic activities
396 (Eilers et al., 2000). When the aggregates were formed in the aquatic environments, a
397 change in bacterial production and respiration occurred with a concurrent change in the
398 bacterial community structure (Grossart and Ploug, 2000). Therefore, DMA amendment
399 to lake water samples seems to activate the DMA-degrading bacteria and/or increase the
400 microbial biomass on the aggregates, promoting the DMA-biodegradation activities.
401 Among all 10 kinds of chemical substrates supplemented to the lake water
402 samples including DMA, the lake water samples including KNO_3 and $NaHCO_3$ at
403 concentrations of 4.0 g L^{-1} completely degraded DMA for 21 days of incubation, while
404 the lake water samples including the other 8 kinds of chemical substrates indicated no
405 significant rates of DMA degradation on the 21st day (Table 3). The anaerobic reduction
406 of nitrate, such as KNO_3, to dinitrogen by nitrate-reducing bacteria was coupled to the
407 oxidation of organic matter (Zumft, 1997). Furthermore, denitrifying bacteria formed
408 nitrogen through anaerobic oxidation of ammonium coupled to nitrate reduction,
409 suggesting that nitrate would promote the growth of denitrifying bacteria (Kniemeyer et
410 al. 1999). The denitrification activities, which occurred in the organic aggregates, might

411 be induced by KNO_3 amendment and contribute to the DMA-degradation process. On
412 the other hand, under anaerobic conditions in aquatic environments, $NaHCO_3$ and
413 hydrogen were converted to methane by methane-synthetic bacteria, whereas the
414 elimination of hydrogen induced the degradation of complex organic matter (McInerney
415 et al., 1979). Zehnder reported that methane-synthetic bacteria would remove the
416 methyl-group from methane compounds and reoxidize methane to carbon dioxide,
417 which is involved in the anaerobic methane oxidization process of the natural
418 environment (Zehnder and Brock, 1979). Probably, anaerobic microbial reactions
419 relating to the methyl-transport and nitrate-reduction processes on organic aggregates
420 are expected to be optimal for converting DMA to inorganic arsenic. Furthermore, the
421 nitrogen and methane cycling on organic aggregates is expected to influence the
422 organoarsenic-compounds mineralization that are key process for understanding the
423 arsenic cycles in aquatic environments.
424
425 **Acknowledgements**
426
427 This research was supported by a Grant-in-Aid for the Encouragement of Young
428 Scientists (22681005) from the Ministry of Education, Science, Sports and Culture. The
429 language has been by English-speaking editor, A. Treadway, in Hot-Line.
430
431 **References**
432
433 Anderson, L. C. D., Bruland, K. W., 1991. Biogeochemistry of arsenic in Natural

waters: The importance of methylated species. Environ. Sci. Technol. 25, 420-427.

Andreae, M. O., 1979. Arsenic speciation in seawater and interstitial waters: The influence of biological-chemical interactions on the chemistry of a trace element. Limnol. Oceanogr. 24, 440-452.

Braman, R. S., Foreback, C. C., 1973. Methylated forms of arsenic in the environment. Science 182, 1247-1249.

Caron, D. A., Davis, P. G., Madin, L. P., Sieburth, J. M., 1982. Heterotrophic bacteria and bacterivorous protozoa in oceanic macroaggregates. Science 218, 795-796.

Crump, B. C., Armbrust, E. V., Baross, J. A., 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. 65, 3192-3204.

Cullen, W. R., Reimer, K. J., 1989. Arsenic speciation in the environment. Chem. Rev. 89, 713-764.

Eilers, H., Pernthaler, J., Amann, R., 2000. Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Appl. Environ. Microbiol. 66, 4634-4640.

Gao, S., Burau, R.G., 1997. Environmental factors affecting rates of arsine evolution from mineralization of arsenicals in soil. J. Environ. Qual. 26, 753-763.

Ghiglione, J. F., Mevel, G., Pujo-Pay, M., Mousseau, L., Lebaron, P., Goutx, M., 2007. Diel and seasonal variations in abundance, activity, and community structure of particle-attached and free-living bacteria in NW Mediterranean Sea. Microbial. Ecol. 54, 217-231.

Grossart, H. P., Ploug, H., 2000. Bacterial production and growth efficiencies: Direct

measurements on riverine aggregates. Limnol. Oceanogr. 45, 436-445.

Hasegawa, H., Sohrin, Y., Seki, K., Sato, M., Norisuye, K., Naito, K., Matsui, M., 2001. Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. Chemosphere 43, 265-272.

Howard, A. G., Comber, S. D. W., 1989. The discovery of hidden arsenic species in coastal waters. Appl. Organomet. Chem. 3, 509-514.

Jenkins, R. O., Ritchie, A. W., Edmonds, J. S., Goessler, W., Molenat, N., Kuehnelt, D., Harrington, C. F., Sutton, P. G., 2003. Bacterial degradation of arsenobetaine via dimethylarsinoylacetate. Arch. Microbiol. 180, 142-150.

Kaise, T., Hanaoka, K., Tagawa, S., 1985. The formation of trimethylarsine oxide from arsenobetaine by biodegradation with marine microorganisms. Chemosphere 16, 2551-2558.

Khokiattiwong, S., Goessler, W., Pedersen, S. N., Cox, R., Francesconi, K. A., 2001. Dimethylarsinoylacetate from microbial demethylation of arsenobetaine in seawater. Appl. Organomet. Chem. 15, 481-489.

Kniemeyer, O., Probian, C., Rossello-Mora, R., Harder J., 1999. Anaerobic mineralization of quaternary carbon atoms: isolation of denitrifying bacteria on dimethylmalonate. Appl. Environ. Microbiol. 65, 3319-3324

Lehr, C. R., Polishchuk, E., Radoja, U., Cullen, W. R., 2003. Demethylation of methylarsenic species by *Mycobacterium neoaurum*. Appl. Organomet. Chem. 17, 831-834.

Maki, T., Takeda, N., Hasegawa, H., Ueda, K., 2006. Isolation of monomethylarsonic acid (MMA)-mineralizing bacteria from arsenic contaminated soils of Island

480 Ohkunoshima. Appl. Organomet. Chem. 20, 538-544.
481 Maki, T., Hirota, W., Ueda, K., Hasegawa, H., Rahman, M. A., 2009. Seasonal
482 dynamics of biodegradation activities for dimethylarsinic acid (DMA) in Lake
483 Kahokugata. Chemosphere 77, 36-42.
484 McInerney, M. J., Bryant, M. P., Pfennig, N., 1979. Anaerobic bacterium that degrades
485 fatty acids in syntrophic association with methanogens. Arch. Microbiol. 122,
486 129-135.
487 Mostajir, B., Dolan, J. R., Rassoulzadegan, F., 1995. A simple method for the
488 quantification of a class of labile marine pico-and nano-sized detritus: DAPI Yellow
489 Particles (DYP). Aquat. Microb. Ecol. 9, 259-266.
490 Neumann, R. B., Ashfaque, K. N., Badruzzaman, A. B. M., Ali, M. A., Shoemaker, J.
491 K., Harvey, C. F., 2010. Anthropogenic influences on groundwater arsenic
492 concentrations in Bangladesh. Nature Geosci. 3, 46-52.
493 Oremland, R. S., 1979. Methanogenic activity in plankton samples and fish intestines: A
494 mechanism for in situ methanogenesis in oceanic surface waters. Limnol Oceanogr.
495 24, 1136-1141.
496 Oremland, R. S., Stolz, J. F., 2003. The ecology of arsenic. Science 300, 939-944.
497 Quinn, J. P., McMullan, G., 1995. Carbon-arsenic bond cleavage by a newly isolated
498 gram-negative bacterium, strain ASV2. Microbiol. 141, 721-725.
499 Russell, W. C., Newman, C., Williamson, D. H., 1974. A simple cytochemical technique
500 for demonstration of DNA in cells infected with mycoplasms and viruses. Nature
501 253, 461-462.
502 Sanders, J. G., 1979. Microbial role in the demethylation and oxidation of methylated

503 arsenicals in seawater. Chemosphere 8, 135-137.
504 Sohrin, Y., Matsui, M., Kawashima, M., Honjo, M., Hasegawa, H., 1997. Arsenic
505 biogeochemistry affected by eutrophication in lake Biwa, Japan. Environ. Sci.
506 Technol. 31, 2712-2720.
507 Unanue, M., Azúa, I., Arrieta, J. M., Labirua-Iturburu, A., Egea, L., Iriberri, J., 1998.
508 Bacterial colonization and ectoenzymatic activity in phytoplankton-derived model
509 particles: Cleavage of peptides and uptake of amino acids. Microbial. Ecol. 35,
510 136-146.
511 Zehnder, A. J., Brock, T. D., 1979. Methane formation and methane oxidation by
512 methanogenic bacteria. J. Bacteriol. 137, 420-432.
513 Zumft, W. G., 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol.
514 Biol. Rev. 61, 533-616.
515
516

517 **Figure legends**

518

519 Fig. 1 Changes in the numbers of free-living bacterial cells (closed circles), yellow
520 fluorescing particles without bacterial particles (open circles), yellow fluorescing
521 particles <5.0 μm with bacterial colonization (open triangles), and yellow fluorescing
522 particles >5.0 μm with bacterial colonization particles (open squares) in lake water
523 samples to which 1 μmol L^{-1} of DMA was added. These particles were counted using
524 epifluorescence microscopic observation with the DAPI staining technique. Each
525 particle count was performed using triplicate bottles.

526

527 Fig. 2 Changes in the concentrations of arsenic compounds in lake water samples with
528 or without filtration to which 1 μmol L^{-1} of DMA was added. Lake water samples
529 without filtration (a) and those filtrated through polycarbonate filters with pore sizes of
530 10 μm (b), 5.0 μm (c), 1.2 μm (d), and 0.22 μm (e) were used. The lake water samples
531 were incubated at 20 ºC under anaerobic and dark conditions. Open circles, closed
532 circles, and closed triangles indicate the abundance of inorganic arsenic, DMA, and
533 MMA, respectively. Each experiment was performed using triplicate bottles.

534

535 Fig. 3 Changes in the concentrations of arsenic compounds in DMA-amended lake
536 water samples to which DMA was added at several concentrations of 1 μmol L^{-1} (a), 5
537 μmol L^{-1} (b), 10 μmol L^{-1} (c), and 50 μmol L^{-1} (d) after 28 days of amendment with 1
538 μmol L^{-1} of DMA and in unamended lake water samples to which DMA was added at a
539 concentration of 10 μmol L^{-1} (e). The lake water samples were incubated at 20 ºC under

anaerobic and dark conditions. Open circles, closed circles, and closed triangles indicate the abundance of inorganic arsenic, DMA, and MMA, respectively. Each experiment was performed in triplicate. The concentrations of inorganic arsenic on day 0 were initialized to 0 μmol L^{-1} by eliminating amounts of inorganic arsenic carried by the first DMA-amendment treatment.

Table 1 Concentrations of inorganic arsenic and DMA in the natural lake water samples, and the lake water samples which were treated for removing microbial activities .*[1]

Incubation time (day)	Untreatment		Autoclave[*2]		Antibiotics addition[*3]		NaN_3 addition[*4]		Filtration[*5]	
	inorganic arsenic	DMA	inorganic arsenic	DMA	inorganic arsenic	DMA	inorganic arsenic	DMA	inorganic arsenic	DMA
0	<10	950 ± 42	<10	958 ± 3	<10	929 ± 46	<10	950 ± 34	<10	934 ± 55
14	<10	932 ± 79	<10	982 ± 32	<10	895 ± 60	<10	920 ± 82	<10	907 ± 67
21	41 ± 13	919 ± 47	<10	900 ± 44	<10	926 ± 75	<10	881 ± 44	<10	951 ± 58
28	912 ± 66	<10	<10	920 ± 75	<10	984 ± 91	<10	897 ± 65	<10	874 ± 47
56	937 ± 44	<10	<10	901 ± 72	<10	923 ± 55	<10	943 ± 8	<10	887 ± 83

***1 All values are given in nmol L^{-1} and the standard error of the mean for triplicate bottles. The lake water samples spiked with DMA were incubated under anaerobic and dark condition at 20 ºC for 21 days**

***2 Lake water was autoclaved at 120 ºC for 20 minutes.**

***3 Antibiotics mixture was added to lake water at a each final concentration of 10 mg L^{-1}.**

***4 NaN_3 was added to lake water at a final concentration of 10 mg L^{-1}.**

***5 Lake water was filtrated with 0.02 µm polycarbonatefilter.**

Table 2. Concentrations of inorganic arsenic and DMA in the DMA-amended and unamended lake water samples and the lake water samples including DMA-amended aggregates.*[1]

Incubation time (day)	DMA amended lake water*[2]		>5.0 μm*[3]		1.2-5.0 μm*[3]		<1.2um*[3]		unamended lake water*[4]	
	inorganic arsenic*[5]	DMA	inorganic arsenic	DMA	inorganic arsenic	DMA	inorganic arsenic	DMA	inorganic arsenic	DMA
0	964	907	<10	923	<10	933	<10	923	<10	907
7	1715 (751)	96	753	111	678	122	<10	900	<10	938
14	1836 (876)	<10	932	<10	833	<10	117	615	280	600
21	1838 (874)	<10	923	<10	865	<10	203	500	849	<10
28	1856 (892)	<10	888	<10	919	<10	890	<10	915	<10

***1 All values are given in nmol L^{-1}. The lake water samplesspiked with DMA were incubated under anaerobic and dark condition at 20 ºC.**

***2 The lake water samples used in this experiment were incubated with DMA for 28 days.**

filters .

***4 The lake water samples that were incubated without DMA for 28 days.**

***5 The values in parentheses showed the increase concentrtaions of inorganic arsenic from the 0 day.**

Table 3 Concentrations of inorganic arsenic and DMA in the lake water samples, to which DMA and some chemical subtrates were added.[a]

Chemical subtrates	Concentrations of chemical substrates (g L^{-1})	inorganic arsenic	DMA
Control	-	<10	973 ± 173
$NaHCO_3$	1.0	631 ± 150	284 ± 162
	4.0	926 ± 11	<10
	10	305 ± 265	627 ± 117
KNO_3	1.0	661 ± 147	206 ± 105
	4.0	953 ± 49	<10
	10	98.4 ± 22.1	741 ± 58
Na_2SO_4	4.0	199 ± 42	745 ± 82
$MgSO_4$	4.0	154 ± 31	845 ± 26
$CuSO_4 \cdot 5H$	4.0	198 ± 22	821 ± 44
$FeSO_4 \cdot 7H_2$	4.0	117 ± 27	768 ± 88
CH_3OONa	4.0	<10	939 ± 23
Glucose	1.0	<10	906 ± 39
Yeast extra	1.0	<10	963 ± 20
L-cystin	1.0	<10	927 ± 26

[a] All values are given in nmol L^{-1} and the standard error of the mean for triplicate bottles. The lake water samples, which were spiked with DMA at final concentrations of 978 ± 63 nmol L^{-1}, were incubated under anaerobic and dark condition at 20 °C for 21 days.

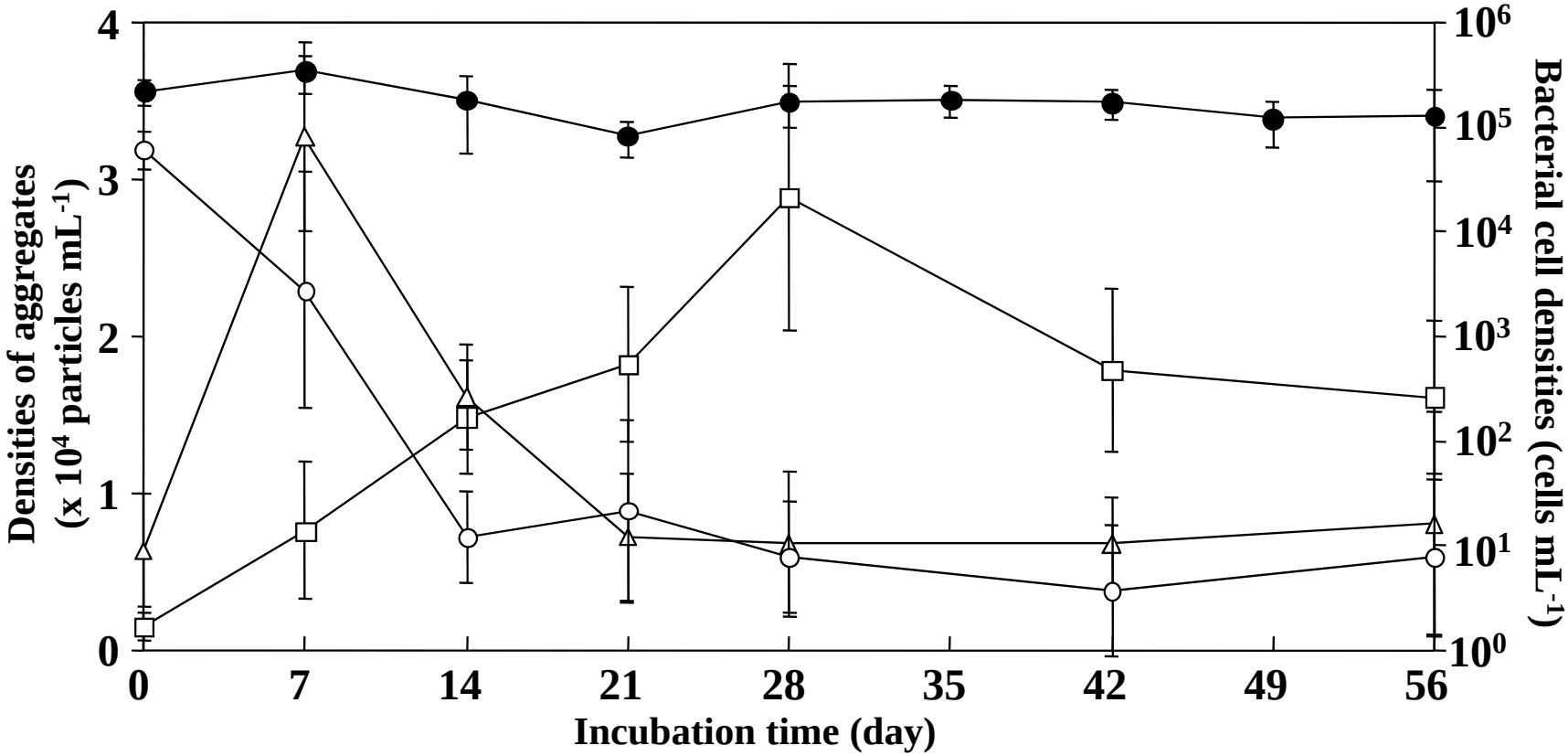

Fig. 1 T. Maki et al.

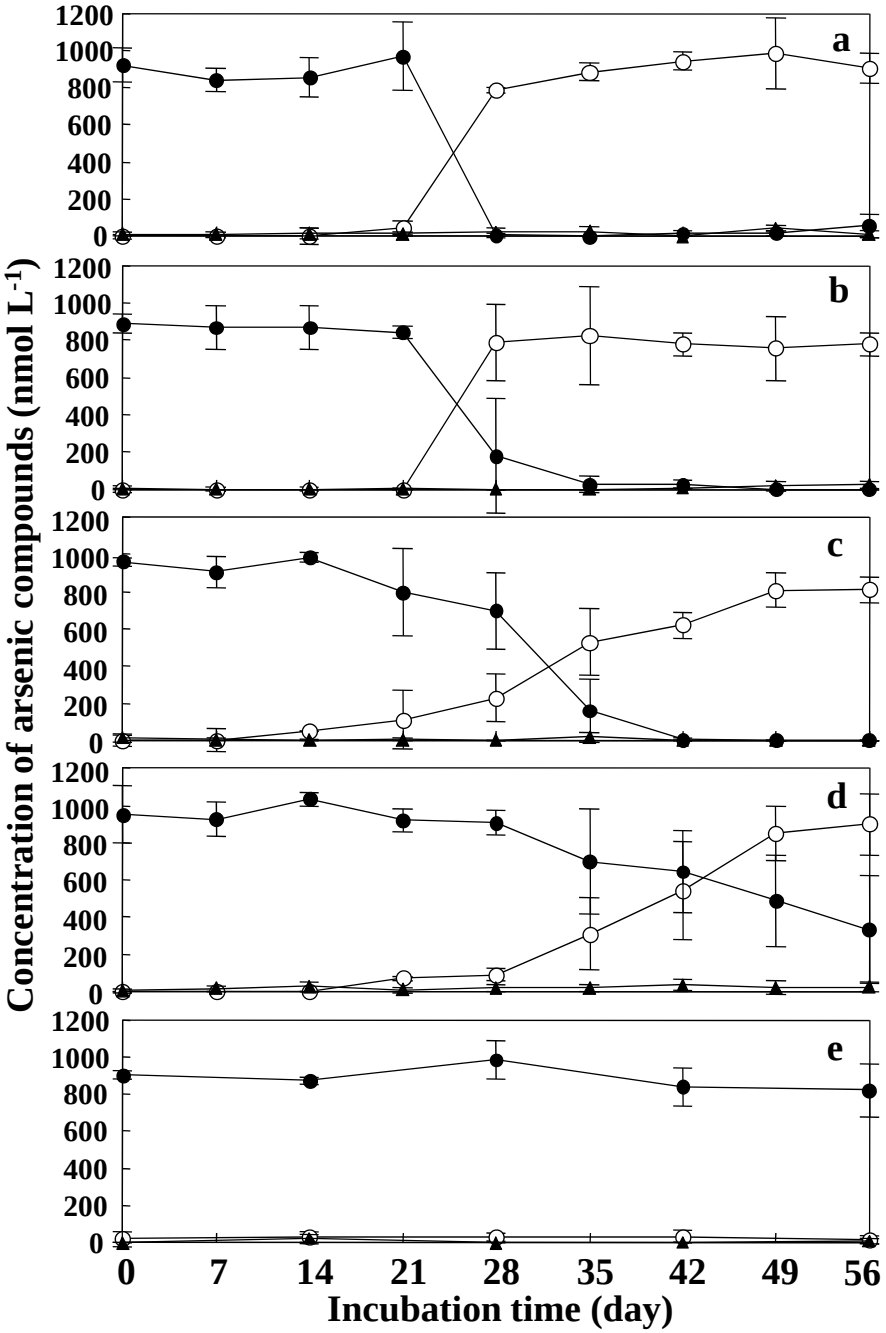

Fig. 2 T. Maki et al.

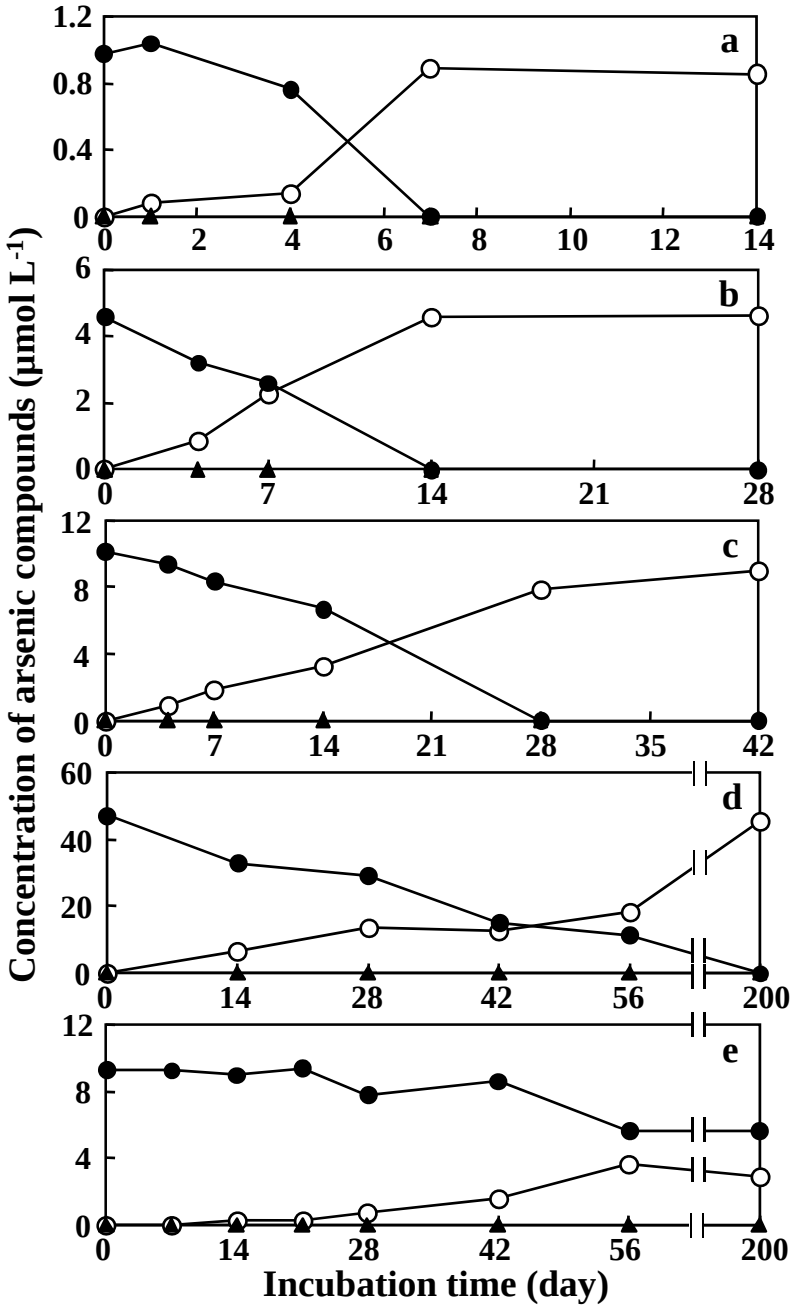

Fig. 3 T. Maki et al.