

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

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1 Title:

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5 Running head:

6 **Biodegradation of dimethylarsinic acid by aggregates**

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19

20 **Abstract**

21

22 Aquatic arsenic cycles mainly depend on microbial activities that change the  
23 arsenic chemical forms and influence human health and organism activities. The  
24 microbial aggregates degrading organic matter are significantly related to the turnover  
25 between inorganic arsenic and organoarsenic compounds. We investigated the effects of  
26 microbial aggregates on organoarsenic mineralization in Lake Kahokugata using lake  
27 water samples spiked with dimethylarsinic acid (DMA). The lake water samples  
28 converted  $1 \mu\text{mol L}^{-1}$  of DMA to inorganic arsenic for 28 days only under anaerobic and  
29 dark conditions in the presence of microbial activities. During the DMA mineralization  
30 process, organic aggregates  $>5.0 \mu\text{m}$  with bacterial colonization increased the densities.  
31 When the organic aggregates  $>5.0 \mu\text{m}$  were eliminated from the lake water samples  
32 using filters, the degradation activities were reduced. DMA in the lake water would be  
33 mineralized by the microbial aggregates under anaerobic and dark conditions. Moreover,  
34 DMA amendment enhanced the degradation activities in the lake water samples, which  
35 mineralized  $50 \mu\text{mol L}^{-1}$  of DMA. The DMA-amended aggregates  $>5.0 \mu\text{m}$  completely  
36 degraded  $1 \mu\text{mol L}^{-1}$  of DMA with a shorter incubation time of 7 days. The supplement  
37 of  $\text{KNO}_3$  and  $\text{NaHCO}_3$  to lake water samples also shortened the DMA degradation  
38 period. Presumably, the bacterial aggregates involved in the chemical heterotrophic  
39 process would contribute to the DMA-biodegradation process in Lake Kahokugata,  
40 which is induced by the DMA amendment.

41

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

43 **Introduction**

44

45 The arsenic species in aquatic ecosystems changed their chemical forms mainly  
46 dependently on the microbial activities that metabolize the arsenic species, and some  
47 arsenic chemical forms are known to endanger human health and organism activities at  
48 high concentrations (Cullen and Reimer, 1989, Neumann et al., 2010). Among the  
49 variety of arsenic species, arsenate, arsenite, and methyl arsenic compounds dominate in  
50 both fresh water and seawater environments (Oremland and Stolz, 2003).  
51 Microorganisms, such as phytoplankton (microalgae) and bacteria, methylate arsenate  
52 into monomethylarsonic acid ( $\text{CH}_3\text{AsO}(\text{OH})_2$ ; MA(V)) and dimethylarsinic acid  
53 ( $(\text{CH}_3)_2\text{AsO}(\text{OH})$ ; DMA(V)) and subsequently convert the methyl arsenic compounds  
54 to more complex organoarsenic compounds, such as arsenobetain and arsenosugar  
55 (Howard and Comber, 1989). However, a significant positive correlation between the *in*  
56 *situ* amounts of chlorophyll a (the biomass of microalgae) and those of organoarsenic  
57 compounds has not been found, because the environmental degradation of  
58 organoarsenic compounds by bacteria had led to this inconsistency (Sohrin et al., 1997).

59 The dominant chemical forms of arsenic compounds in a number of lakes and  
60 estuaries have been reported to change seasonally by the degradation and production of  
61 organoarsenic compounds (Anderson and Bruland, 1991, Maki et al., 2009). A few  
62 reports have described that bacterial consortia in marine environments could degrade  
63 the amended organoarsenic compounds (Sanders, 1979, Kaise et al., 1985) and that  
64 bacterial population associated with crabs (Khokiattiwong et al., 2001) and mussels  
65 (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

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

99

## 100 **Materials and Methods**

101

### 102 **Sampling at Lake Kahokugata**

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

112 of Lake Kahokugata in polycarbonate bottles were used for several culture experiments,  
113 in which we estimated the DMA-biodegradation activities of lake water under several  
114 experimental treatments and the microbial dynamics during the DMA-degradation  
115 process. The concentrations of inorganic arsenic and organoarsenic compounds in the  
116 lake water without the addition of DMA were stable below 20 nmol L<sup>-1</sup> during the entire  
117 experiment.

118

### 119 **Influences of the microbial activities on DMA biodegradation in lake water**

120 Lake water samples collected in polycarbonate bottles from Lake Kahokugata on  
121 29 August 2008 were used to investigate the DMA-biodegradation activities in lake  
122 water samples incubated under anaerobic and dark conditions. Fifteen polycarbonate  
123 bottles (500 mL) were filled up with lake water and transferred to our laboratory. Within  
124 2 hours of sampling, 500 μL of a 1 mmol L<sup>-1</sup> DMA (Nacalai Tesque, Kyoto, Japan)  
125 solution was added into 3 bottles, and the final concentration of DMA was adjusted to 1  
126 μmol L<sup>-1</sup>. The bottles were incubated under anaerobic and dark conditions. To produce  
127 the anaerobic conditions, the air phases in the bottles were kept at the lowest possible  
128 level, and the lake water samples were purged with nitrogen (100 mL min<sup>-1</sup>) for 0.5  
129 hours. The bottles were incubated in a temperature-controlled room (20 °C) under dark  
130 conditions by covering the bottles with aluminum foil. The anaerobic and dark  
131 condition of incubation was optimal for DMA-degradation in lake water (Maki et al.,  
132 2009).

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

135 water sample was autoclaved at 120 °C for 20 minutes; an antibiotic mixture was added  
136 to each sample of lake water at a final concentration of 10 mg L<sup>-1</sup>; sodium azide was  
137 added to each sample of lake water at a final concentration of 10 mg L<sup>-1</sup>; 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<sup>-1</sup> DMA were  
141 incubated at 20 °C 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<sup>-1</sup>. Triplicate bottles were prepared for each sample of filtrate,  
154 and incubated at a temperature of 20 °C 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



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

159 For investigating the activation of DMA degradation by the DMA amendment,  
160 the lake water samples collected on 22 May 2008 were spiked with  $1 \mu\text{mol L}^{-1}$  of DMA  
161 and amended at  $20 \text{ }^\circ\text{C}$  under anaerobic and dark conditions for 28 days (DMA-amended  
162 lake water sample). DMA in the DMA-amended lake water samples was completely  
163 converted to inorganic arsenic within 28 days of incubation. The 500 mL  
164 DMA-amended lake water samples were sequentially filtrated through a series of  
165 polycarbonate filters with pore sizes of 5.0 and  $1.2 \mu\text{m}$  (Millipore, Tokyo, Japan). The  
166 aggregates on the 5.0 and  $1.2 \mu\text{m}$  filters were added to 500 mL lake water samples that  
167 were collected on 9 June 2008 and filtrated through a  $0.02 \mu\text{m}$  pore-size polycarbonate  
168 filter. The filtrate with the  $1.2 \mu\text{m}$  filters was also used for the following experiments as  
169 the lake water sample including  $<1.2 \mu\text{m}$  aggregates. Finally, the lake water samples  
170 including the aggregates separated into three fractions:  $> 5.0 \mu\text{m}$ ,  $5.0 \mu\text{m}$  to  $1.2 \mu\text{m}$ , and  
171  $<1.2 \mu\text{m}$  were prepared. One  $\mu\text{mol L}^{-1}$  of DMA was spiked to the lake water samples  
172 including each fraction of aggregates and unfiltrated DMA-amended lake water sample  
173 (500 mL). The water samples were incubated at  $20 \text{ }^\circ\text{C}$  under anaerobic and dark  
174 conditions. At every several days of incubation, the concentrations of arsenic species in  
175 portions (10 mL) of the lake water samples were measured.

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

181 sample). The 4 bottles of DMA-amended lake water samples were then spiked with  
182 DMA at the final concentrations of 1, 5, 10, and 50  $\mu\text{mol L}^{-1}$ , and the bottle of  
183 unamended lake water samples was also spiked with 10  $\mu\text{mol L}^{-1}$  DMA. These bottles  
184 were continuously incubated at 20 °C under anaerobic and dark conditions. At every 7  
185 days after the second DMA spike, portions (10 mL) of the lake water samples were  
186 collected to determine the concentrations of arsenic species.

187

### 188 **Effects of chemical substrates on DMA biodegradation in lake water**

189 The polycarbonate bottles (500 mL) were filled up with lake water samples  
190 collected from Lake Kahokugata on 11 September 2009. The lake water samples were  
191 supplemented with one of 10 kinds of chemical substrates;  $\text{NaHCO}_3$  (final  
192 concentration: 1.0, 4.0, and 10  $\text{g L}^{-1}$ ),  $\text{KNO}_3$  (1.0, 4.0, and 10  $\text{g L}^{-1}$ ),  $\text{Na}_2\text{SO}_4$  (4.0  $\text{g L}^{-1}$ ),  
193  $\text{MgSO}_4$  (4.0  $\text{g L}^{-1}$ ),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (4.0  $\text{g L}^{-1}$ ),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (4.0  $\text{g L}^{-1}$ ),  $\text{CH}_3\text{COONa}$  (4.0  
194  $\text{g L}^{-1}$ ), L-glucose (1.0  $\text{g L}^{-1}$ ), yeast extract (1.0  $\text{g L}^{-1}$ ), or L-cystin (1.0  $\text{g L}^{-1}$ ) (Nacalai  
195 Tesque, Kyoto, Japan). The amendment using  $\text{NaHCO}_3$  and  $\text{KNO}_3$  increase the activities  
196 of methan-synthetic and nitrogen-reducing bacterial consortia in lake water samples.  
197 The addition of  $\text{Na}_2\text{SO}_4$ ,  $\text{MgSO}_4$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  activate the  
198 sulfate-reducing bacterial consortia. The addition of  $\text{CH}_3\text{COONa}$ , L-glucose, yeast  
199 extract, and L-cystin increase the cometabolic activities of several bacterial  
200 communities. Lake water samples without chemical-substrate addition were also  
201 prepared as controls. DMA was added to the lake water samples at a final concentration  
202 of 1  $\mu\text{mol L}^{-1}$ , and each experiment was performed using triplicate bottles. After 21 days  
203 of incubation at 20 °C under anaerobic and dark conditions, the concentrations of

204 arsenic species in the lake water samples were determined.

205

### 206 **Measurements of the arsenic compound concentration**

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

217

### 218 **Observation of particles in lake water samples using epifluorescence microscopy**

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

227 filter was placed on a slide on top of a drop of low-fluorescence immersion oil, a drop  
228 of oil was added and then covered with a cover slide. Slides were examined using an  
229 epifluorescence microscope (Olympus, Tokyo, Japan) under UV excitation. Under the  
230 microscopic observation, the free-living bacterial particles in lake water samples were  
231 observed as coccoid-like or bacillus-like particles with white-blue fluorescence with a  
232 diameter of below 1.0  $\mu\text{m}$ . Furthermore, the particles with yellow fluorescence ranging  
233 from 1.0  $\mu\text{m}$  to about 500  $\mu\text{m}$  diameters were also observed. Among the yellow  
234 fluorescing particles, some of them were colonized with bacterial particles, and the  
235 others were not attached with the bacterial particles. A filter transect was scanned and  
236 the particles on the filter transect, which could be discriminated into free-living bacterial  
237 particles, yellow fluorescing particles without bacterial-particle attachment, and yellow  
238 fluorescing particles with bacterial colonization in two size categories ( $<5.0 \mu\text{m}$  and  
239  $>5.0 \mu\text{m}$ ), were enumerated. Each particle count was performed on 10 microscopic  
240 fields randomly selected.

241

## 242 **Results**

243

### 244 **Influence of the microbial activities on DMA biodegradation in lake water**

245         When the lake water samples were spiked with DMA at a final concentration of  
246 approximately 1  $\mu\text{mol L}^{-1}$  and incubated at 20 °C under anaerobic and dark conditions,  
247 the concentration of DMA at the onset of the experiment decreased from 916  $\text{nmol L}^{-1}$   
248 (average) to the detection limit (avg.) during the first 28 days of incubation (Table 1).  
249 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<sup>-1</sup> at the 28th day and fluctuated the concentration of 900 nmol L<sup>-1</sup> 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  $3.2 \times 10^4$  to  $7.2 \times 10^3$  particles  
262 mL<sup>-1</sup> 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  $6.4 \times 10^3$  to  $3.3 \times 10^4$  particles mL<sup>-1</sup> during the first  
265 7 days and decreased to  $7.2 \times 10^3$  particles mL<sup>-1</sup> on the 21st day, maintaining the order  
266 of  $10^3$  particles mL<sup>-1</sup> until the 56th day. The densities of yellow fluorescing particles  
267 >5.0 μm with bacterial colonization gradually increased from  $1.5 \times 10^3$  to  $2.9 \times 10^4$   
268 particles mL<sup>-1</sup> for 28 days, and fluctuated over densities of  $1.6 \times 10^4$  particles mL<sup>-1</sup> until  
269 the 56th day. The free-living bacterial cells maintained concentrations ranging from  $10^4$   
270 to  $10^5$  cells mL<sup>-1</sup> during the experimental period. The total densities of bacterial particles  
271 colonized on yellow fluorescing particles >5.0 μm were ranged from  $2.0 \times 10^4$  to  $3.2 \times$   
272  $10^5$  particles mL<sup>-1</sup> for 28 days (data not shown), suggesting that the total biomasses of

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

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

285

### 286 **Induction of DMA biodegradation in the DMA-amended lake water samples**

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

296 >5.0  $\mu\text{m}$  was completely converted to inorganic arsenic in 7 days (Table 2). The lake  
297 water samples including aggregates 1.2-5.0  $\mu\text{m}$  or <1.2  $\mu\text{m}$  completely mineralized the  
298 DMA for longer incubation times of 21 days and 28 days, respectively (Table 2).  
299 Consequently, aggregates >5.0  $\mu\text{m}$  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  $\mu\text{mol L}^{-1}$ , 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  $\mu\text{mol L}^{-1}$  of DMA, up to 4  $\mu\text{mol L}^{-1}$  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  $\text{KNO}_3$  and  $\text{NaHCO}_3$   
313 significantly mineralized 1  $\mu\text{mol L}^{-1}$  of DMA on the 21st day of incubation (Table 3). In  
314 the lake water samples supplemented with 4.0  $\text{g L}^{-1}$  of  $\text{KNO}_3$  and  $\text{NaHCO}_3$ , DMA was  
315 remarkably decreased, and inorganic arsenic increased to over 900  $\text{nmol L}^{-1}$  on the 21st  
316 day of incubation. The lake water samples including 4.0  $\text{g L}^{-1}$  of  $\text{KNO}_3$  and  $\text{NaHCO}_3$   
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<sup>-1</sup> KNO<sub>3</sub> and NaHCO<sub>3</sub> 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<sub>3</sub> and NaHCO<sub>3</sub> at proper  
322 concentrations, such as 4.0 g L<sup>-1</sup>, 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  $>5.0\ \mu\text{m}$  with bacterial  
354 colonization increased during the DMA-degradation process (Fig. 1). Moreover, the  
355 lake water samples filtrated through  $>5.0\ \mu\text{m}$  pore-size filters showed similar rates of  
356 DMA biodegradation to unfiltrated lake water samples, while the lake water samples  
357 filtrated through  $<5.0\ \mu\text{m}$  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

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

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

384         In the DMA-amended lake water samples and the lake water samples containing  
385 DMA-amended aggregates  $>5.0 \mu\text{m}$ ,  $1 \mu\text{mol L}^{-1}$  DMA was rapidly converted to  
386 inorganic arsenic in 7 days (Table 2). On the contrary, DMA in the unamended lake  
387 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  $\mu\text{mol L}^{-1}$  of DMA, whereas the unamended lake water sample could mineralize a  
390 maximum of 4  $\mu\text{mol L}^{-1}$  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  $\text{KNO}_3$  and  $\text{NaHCO}_3$  at  
403 concentrations of 4.0  $\text{g L}^{-1}$  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  $\text{KNO}_3$ , 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  $\text{KNO}_3$  amendment and contribute to the DMA-degradation process. On  
412 the other hand, under anaerobic conditions in aquatic environments,  $\text{NaHCO}_3$  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

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426

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430

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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  $<5.0 \mu\text{m}$  with bacterial colonization (open triangles), and yellow fluorescing  
522 particles  $>5.0 \mu\text{m}$  with bacterial colonization particles (open squares) in lake water  
523 samples to which  $1 \mu\text{mol L}^{-1}$  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  $1 \mu\text{mol L}^{-1}$  of DMA was added. Lake water samples  
529 without filtration (a) and those filtrated through polycarbonate filters with pore sizes of  
530  $10 \mu\text{m}$  (b),  $5.0 \mu\text{m}$  (c),  $1.2 \mu\text{m}$  (d), and  $0.22 \mu\text{m}$  (e) were used. The lake water samples  
531 were incubated at  $20 \text{ }^\circ\text{C}$  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  $1 \mu\text{mol L}^{-1}$  (a),  $5$   
537  $\mu\text{mol L}^{-1}$  (b),  $10 \mu\text{mol L}^{-1}$  (c), and  $50 \mu\text{mol L}^{-1}$  (d) after 28 days of amendment with  $1$   
538  $\mu\text{mol L}^{-1}$  of DMA and in unamended lake water samples to which DMA was added at a  
539 concentration of  $10 \mu\text{mol L}^{-1}$  (e). The lake water samples were incubated at  $20 \text{ }^\circ\text{C}$  under

540 anaerobic and dark conditions. Open circles, closed circles, and closed triangles indicate  
541 the abundance of inorganic arsenic, DMA, and MMA, respectively. Each experiment  
542 was performed in triplicate. The concentrations of inorganic arsenic on day 0 were  
543 initialized to 0  $\mu\text{mol L}^{-1}$  by eliminating amounts of inorganic arsenic carried by the first  
544 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 .<sup>\*1</sup>**

Incubati on time (day)	Untreatment		Autoclave <sup>*2</sup>		Antibiotics addition <sup>*3</sup>		NaN <sub>3</sub> addition <sup>*4</sup>		Filtration <sup>*5</sup>	
	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

<sup>\*1</sup> All values are given in nmol L<sup>-1</sup> 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 °C for 21 days

<sup>\*2</sup> Lake water was autoclaved at 120 °C for 20 minutes.

<sup>\*3</sup> Antibiotics mixture was added to lake water at a each final concentration of 10 mg L<sup>-1</sup>.

<sup>\*4</sup> NaN<sub>3</sub> was added to lake water at a final concentration of 10 mg L<sup>-1</sup>.

<sup>\*5</sup> 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.\*<sup>1</sup>**

Incubation time (day)	DMA amended lake water* <sup>2</sup>		>5.0 $\mu\text{m}$ * <sup>3</sup>		1.2-5.0 $\mu\text{m}$ * <sup>3</sup>		<1.2 $\mu\text{m}$ * <sup>3</sup>		unamended lake water* <sup>4</sup>	
	inorganic arsenic* <sup>5</sup>	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  $\text{nmol L}^{-1}$ . The lake water samples spiked with DMA were incubated under anaerobic and dark condition at 20 °C.

\*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 concentrations 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 substrates were added.<sup>a</sup>**

<b>Chemical substrates</b>	<b>Concentrations of chemical substrates (g L<sup>-1</sup>)</b>	<b>inorganic arsenic</b>	<b>DMA</b>
<b>Control</b>	<b>-</b>	<b>&lt;10</b>	<b>973 ± 173</b>
<b>NaHCO<sub>3</sub></b>	<b>1.0</b>	<b>631 ± 150</b>	<b>284 ± 162</b>
	<b>4.0</b>	<b>926 ± 11</b>	<b>&lt;10</b>
	<b>10</b>	<b>305 ± 265</b>	<b>627 ± 117</b>
<b>KNO<sub>3</sub></b>	<b>1.0</b>	<b>661 ± 147</b>	<b>206 ± 105</b>
	<b>4.0</b>	<b>953 ± 49</b>	<b>&lt;10</b>
	<b>10</b>	<b>98.4 ± 22.1</b>	<b>741 ± 58</b>
<b>Na<sub>2</sub>SO<sub>4</sub></b>	<b>4.0</b>	<b>199 ± 42</b>	<b>745 ± 82</b>
<b>MgSO<sub>4</sub></b>	<b>4.0</b>	<b>154 ± 31</b>	<b>845 ± 26</b>
<b>CuSO<sub>4</sub>·5H</b>	<b>4.0</b>	<b>198 ± 22</b>	<b>821 ± 44</b>
<b>FeSO<sub>4</sub>·7H<sub>2</sub></b>	<b>4.0</b>	<b>117 ± 27</b>	<b>768 ± 88</b>
<b>CH<sub>3</sub>OONa</b>	<b>4.0</b>	<b>&lt;10</b>	<b>939 ± 23</b>
<b>Glucose</b>	<b>1.0</b>	<b>&lt;10</b>	<b>906 ± 39</b>
<b>Yeast extra</b>	<b>1.0</b>	<b>&lt;10</b>	<b>963 ± 20</b>
<b>L-cystin</b>	<b>1.0</b>	<b>&lt;10</b>	<b>927 ± 26</b>

<sup>a</sup> All values are given in nmol L<sup>-1</sup> 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<sup>-1</sup>, were incubated under anaerobic and dark condition at 20 °C for 21 days.

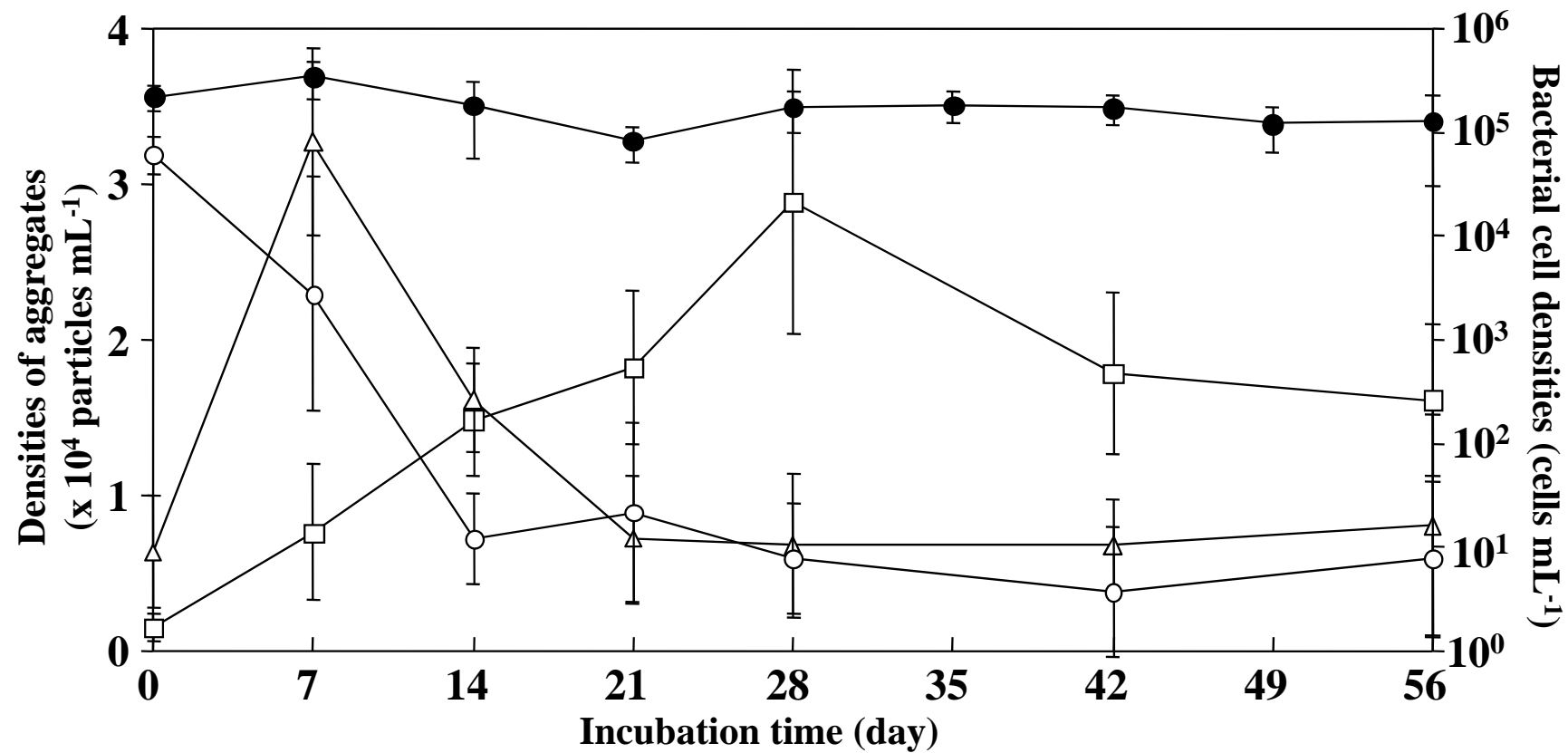


Fig. 1 T. Maki et al.

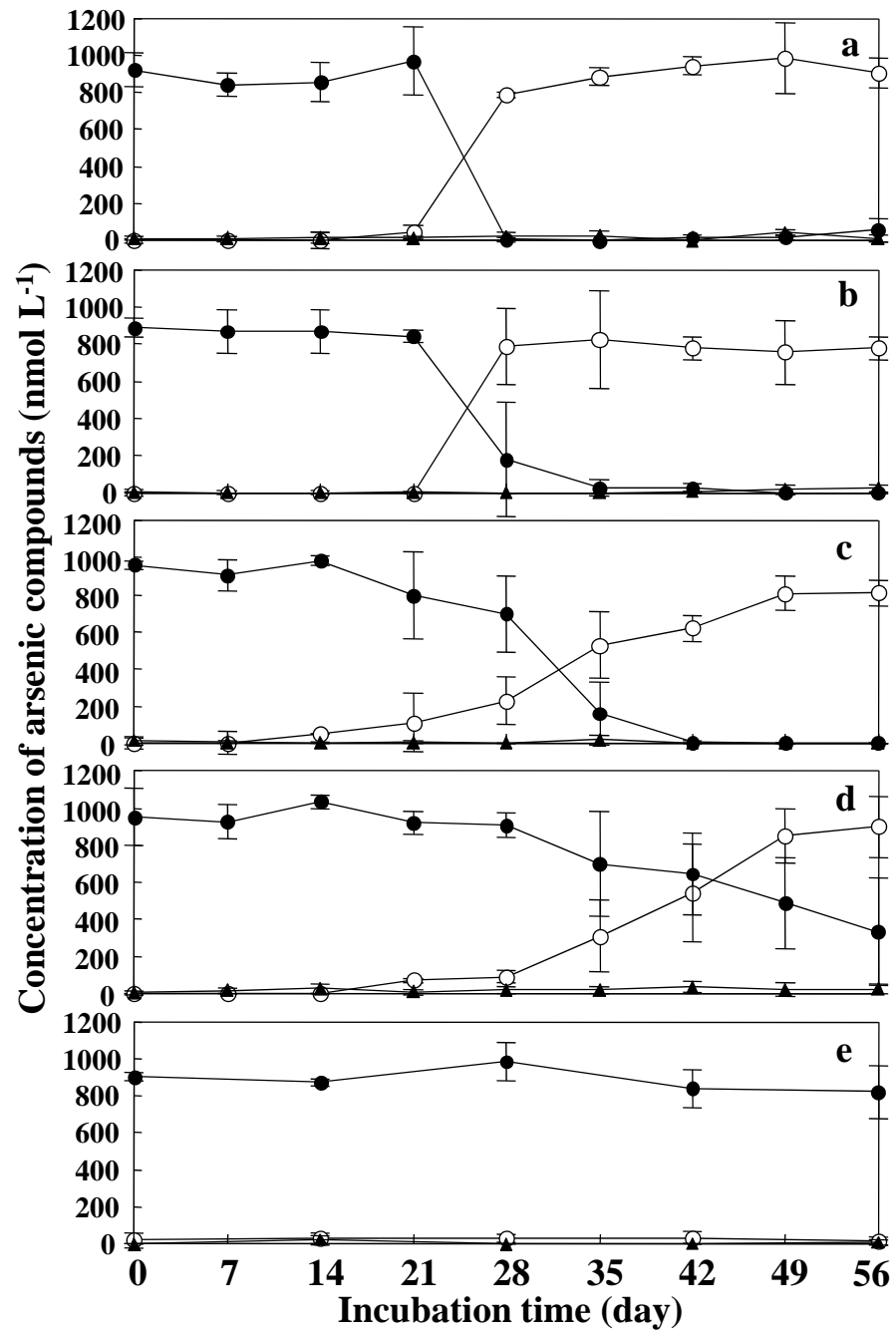


Fig. 2 T. Maki et al.

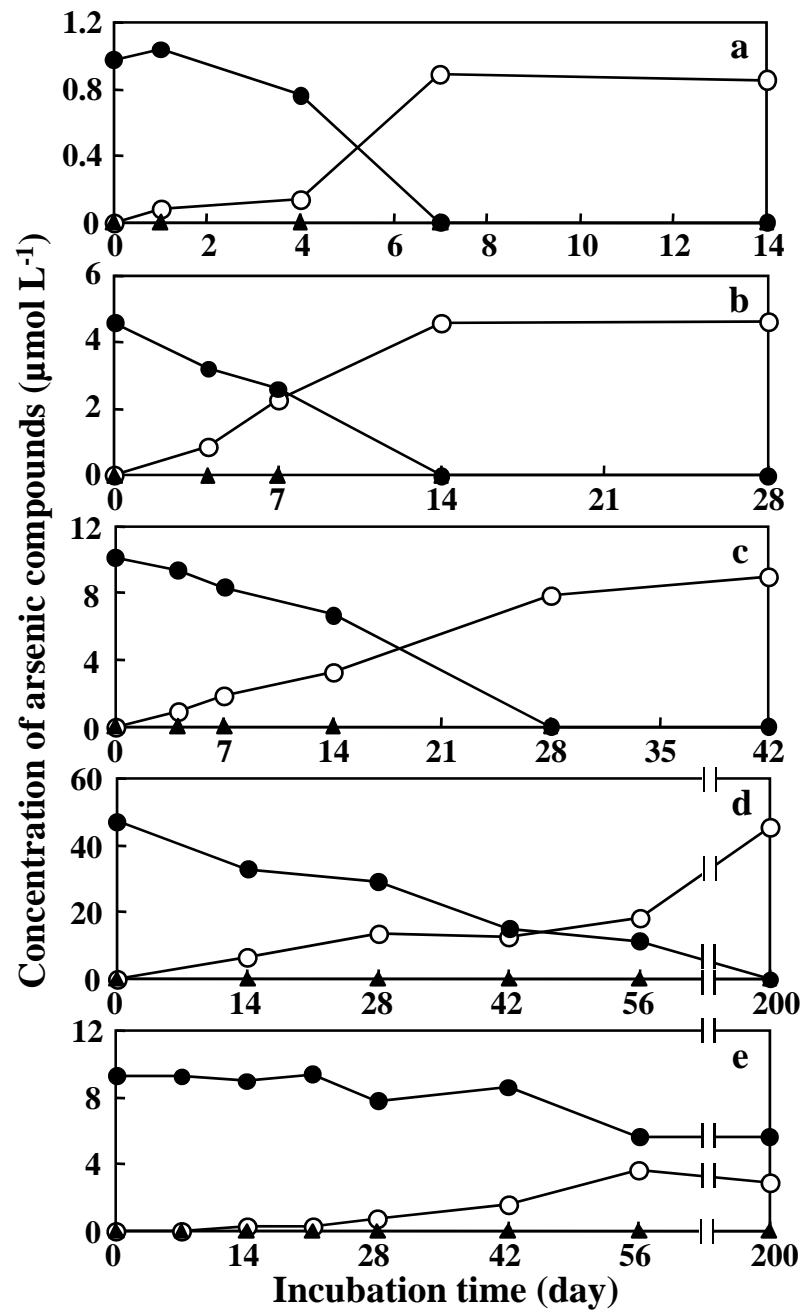


Fig. 3 T. Maki et al.