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

1 Title:

2 **Influence of aggregated particles on biodegradation activities for dimethylarsinic**

3 **acid (DMA) in Lake Kahokugata**

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5 Running head:

6 **Biodegradation of dimethylarsinic acid by aggregates**

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Abstract

20

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 µmol 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 µm with bacterial colonization increased the densities.

31 When the organic aggregates >5.0 µm 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 µmol L^{-1} of DMA. The DMA-amended aggregates >5.0 µm completely

36 degraded 1 µmol L^{-1} of DMA with a shorter incubation time of 7 days. The supplement

37 of KNO_3 and $NaHCO_3$ 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

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.

The several species of bacterial isolates degrading organoarsenic compounds were

obtained from activated sludge (Quinn and McMullan, 1995) and natural environments

(Lehr et al., 2003, Maki et al., 2006). The degradation process of organoarsenic

compounds is worthy of study for understanding the ecological dynamics and

distribution of arsenic compounds in aquatic environments.

In aquatic environments, organic aggregates colonized by heterotrophic

microorganisms are important components in the turnover of both organic and inorganic

matter (Grossart and Ploug, 2000). The bacteria population attached to organic

aggregates has been reported to exhibit higher metabolic activities than free-living

bacterial population in the surrounding waters (Caron et al., 1982, Ghiglione et al.,

2007). Organic aggregates and their surrounding environments are the hot spots of the

microbial process, during which the degradation activities of organic matter are

expanded (Crump et al., 1999). In the organic aggregates where concentration gradients

of oxygen develop, the organic-degradation activities of anaerobic bacteria, such as

denitrifying bacteria and methane-synthetic bacteria, are high (e.g. Oremland 1979).

Our previous study demonstrated that the DMA biodegradation in lake water was

activated only under anaerobic and dark condition, indicating the possibility that the

organic aggregates provide the hot spots for the DMA-degrading microorganisms and

support the DMA-biodegradation (Maki et al. 2009). However, the actual

biodegradation of DMA by organic aggregates with bacterial colonization is still

unknown.

In this study, the influence of aggregates on the DMA-biodegradation process

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 unamened lake water to

91   evaluate the activation of DMA degradation by the amendment. The microbial consocia

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 consocia 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^{-1} to 8.3 mg

108   L^{-1} 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^{-1} 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^{-1} 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^{-1}. 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^{-1}) for 0.5

129   hours. The bottles were incubated in a temperature-controlled room (20 ℃) 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 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

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 µmol L^{-1}, and the bottle of

183   unamended lake water samples was also spiked with 10 µmol L^{-1} DMA. These bottles

184   were continuously incubated at 20 ℃ 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; NaHCO$_3$ (final

192   concentration: 1.0, 4.0, and 10 g L^{-1}), KNO$_3$ (1.0, 4.0, and 10 g L^{-1}), Na$_2$SO$_4$ (4.0 g L^{-1}),

193   MgSO$_4$ (4.0 g L^{-1}), CuSO$_4$·5H$_2$O (4.0 g L^{-1}), FeSO$_4$·7H$_2$O (4.0 g L^{-1}), CH$_3$COONa (4.0

194   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

195   Tesque, Kyoto, Japan). The amendment using NaHCO$_3$ and KNO$_3$ increase the activities

196   of methan-synthetic and nitrogen-reducing bacterial consortia in lake water samples.

197   The additon of Na$_2$SO$_4$, MgSO$_4$, CuSO$_4$·5H$_2$O, and FeSO$_4$·7H$_2$O activate the

198   sulfate-reducing bacterial consortia. The addition of CH$_3$COONa, L-g1ucose, 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 µmol L^{-1}, and each experiment was performed using triplicate bottles. After 21 days

203   of incubation at 20 ℃ under anaerobic and dark conditions, the concentrations of

204   arsenic species in the lake water samples were determined.

205

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 µm 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 ˚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 nmol L^{-1}.

217

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 µmol

221   L^{-1}. For 56 days of incubation at 20 ℃ 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 µg

225   mL^{-1} for 15 min and filtrated through a 0.22 µm 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 µm. Furthermore, the particles with yellow fluorescence ranging

233 from 1.0 µm to about 500 µm 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 µm and

239 >5.0 µm), 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 µmol 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 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^{-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

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 µmol L^{-1} of DMA, the lake water samples filtrated through a 10 µm

276 pore-size filter degraded 90 % of DMA and accumulated inorganic arsenic at a

277 concentration of 860 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 µm and 1.2 µm 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 µm. In the lake water samples filtrated

283 through the 0.22 µm 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 µmol 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 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 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

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 µm with bacterial colonization increased (Fig. 1),

370   suggesting that the bacteria form their colonies on organic aggregates >5.0 µm during

371   the incubation period. Furthermore, the aggregates >5.0 µm 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 µm 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 µm, 1 µmol 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.

17

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**

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431   **References**

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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

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 μmol 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 .[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$_2$SO$_4$	4.0	199 ± 42	745 ± 82
MgSO$_4$	4.0	154 ± 31	845 ± 26
CuSO$_4$·5H	4.0	198 ± 22	821 ± 44
FeSO$_4$·7H$_2$	4.0	117 ± 27	768 ± 88
CH$_3$OONa	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 ºC for 21 days.
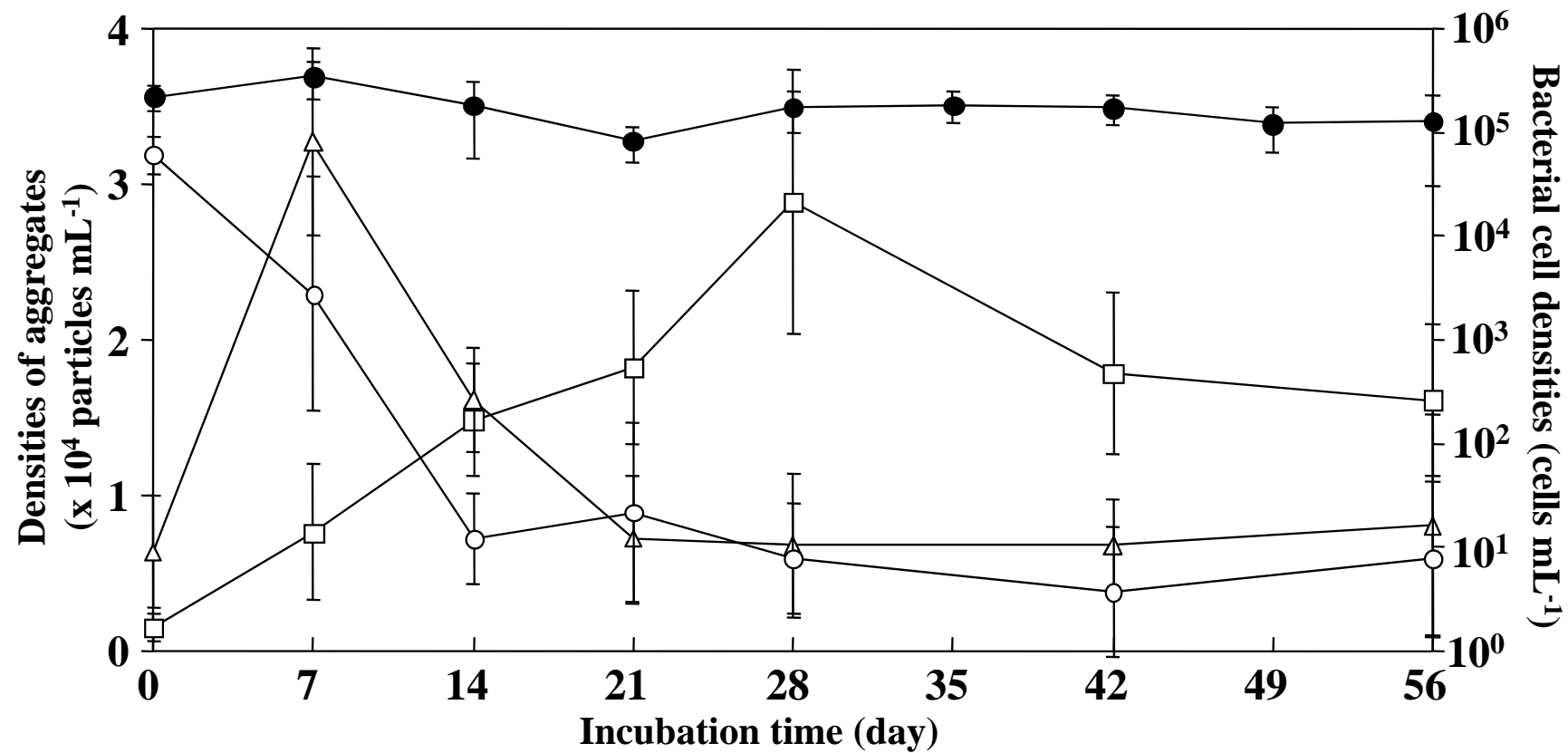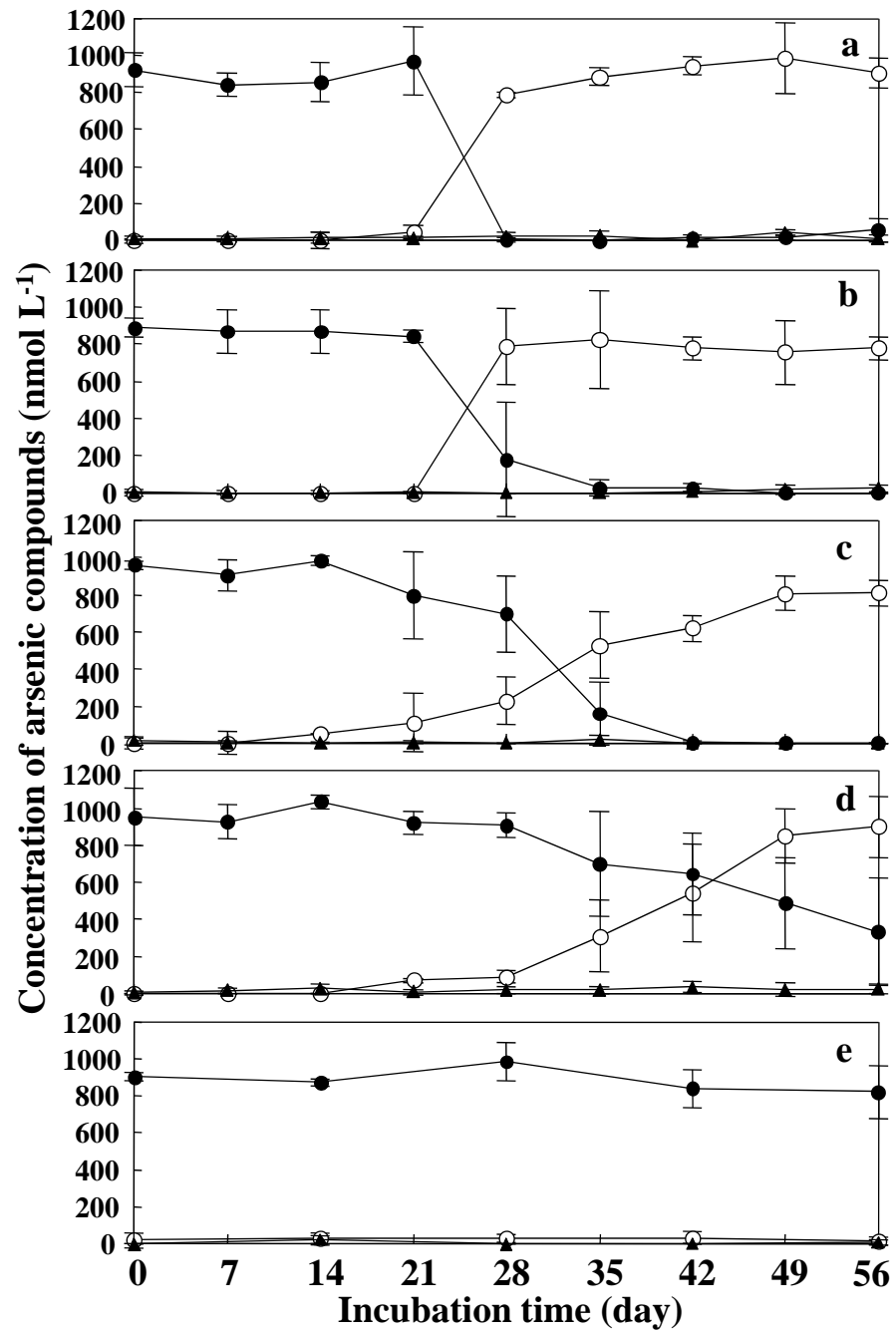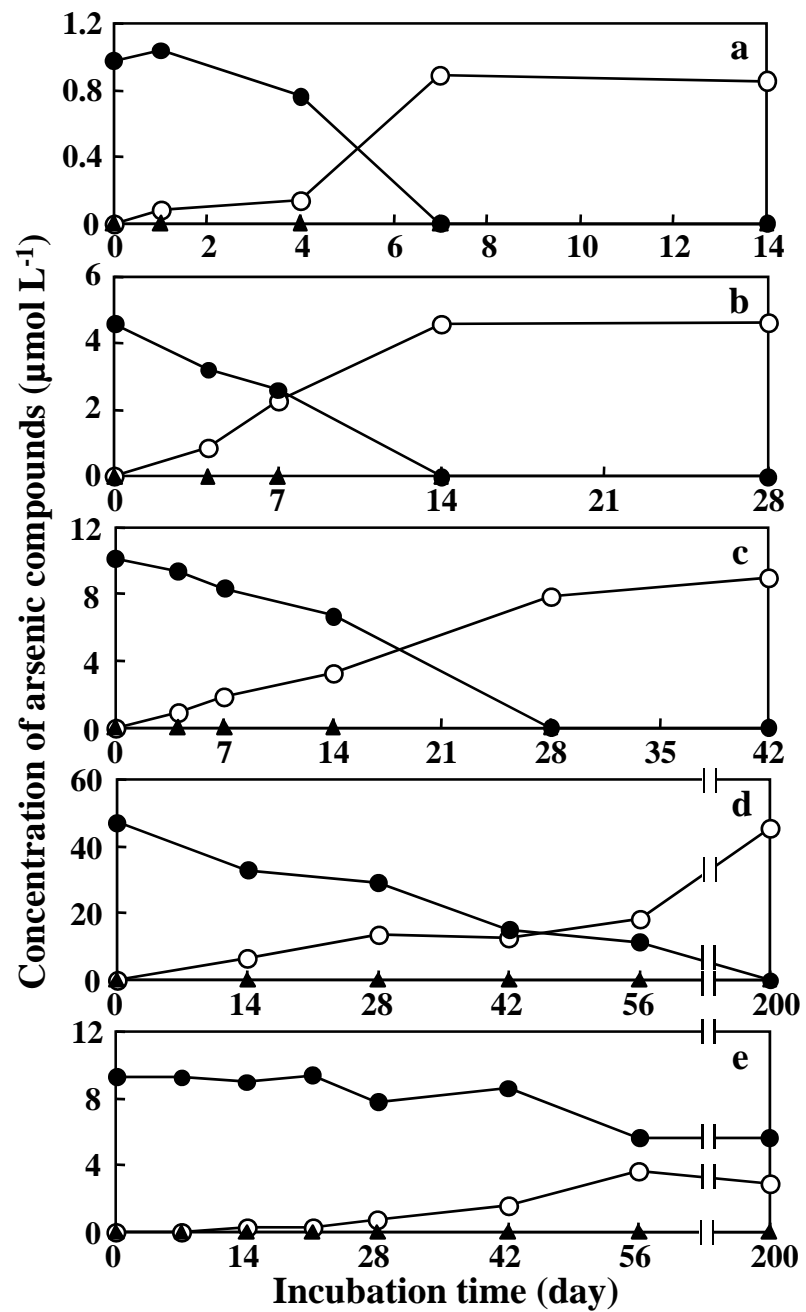
Fig. 1 T. Maki et al.

Fig. 2 T. Maki et al.

Fig. 3 T. Maki et al.