

Oxidation of arsenite by self-regenerative bioactive birnessite in a continuous flow column reactor

Nishi, Ryohei

Department of Earth Resources Engineering, Faculty of Engineering, Kyushu University

Kitjanukit, Santisak

Department of Earth Resources Engineering, Faculty of Engineering, Kyushu University

Nonaka, Kohei

Department of Earth Resources Engineering, Faculty of Engineering, Kyushu University

Okibe, Naoko

Department of Earth Resources Engineering, Faculty of Engineering, Kyushu University

<https://hdl.handle.net/2324/4739237>

出版情報 : Hydrometallurgy. 196 (105416), 2020-09. Elsevier

バージョン :

権利関係 :

1 *Hydrometallurgy (IBS2019 Special Issue)*

2

3 Title: Oxidation of arsenite by self-regenerative bioactive birnessite in a continuous flow column reactor

4

5 Ryohei Nishi, Santisak Kitjanukit, Kohei Nonaka and Naoko Okibe*

6

7 Department of Earth Resources Engineering, Faculty of Engineering, Kyushu University, 744 Motoooka,
8 Nishi-ku, Fukuoka 819-0395, Japan

9

10 *Corresponding author

11 Tel. and Fax: +81 92 802 3312

12 E-mail address: okibe@mine.kyushu-u.ac.jp (Naoko OKIBE)

13

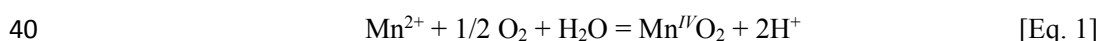
14 **Keywords:** arsenite; oxidation; birnessite, Mn-oxidizing bacteria, continuous column reactor

15 **Abstract**

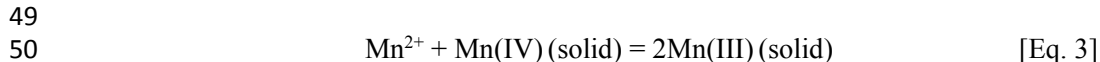
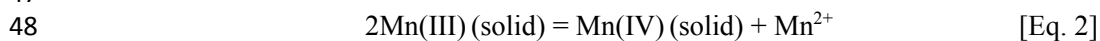
16 Naturally occurring manganese (Mn) oxide, biogenic birnessite $((\text{Na}, \text{Ca}, \text{K})_{0.5} \text{Mn}^{\text{III}, \text{IV}} \text{O}_4 \cdot 1.5 \text{H}_2\text{O})$, is
17 involved in the geochemical cycling of variety of metals including arsenic (As). This natural reaction was
18 exploited in this study to develop a sustainable oxidation treatment process of As(III) to the less soluble
19 (and less toxic) As(V). It is known that the birnessite surface becomes passivated during As(III) oxidation,
20 which quickly decreases its reactivity. The cycle batch test and the following XANES (X-ray absorption
21 near-edge structure) analysis in this study confirmed that combining chemical As(III) oxidation by
22 birnessite with simultaneous birnessite regeneration by Mn-oxidizing microorganisms (*Pseudomonas* sp.
23 SK3) can avoid passivation of Mn^{III}-precipitates and enables continuous As(III) oxidation while increasing
24 the AOS (average oxidation state) of birnessite. This chemical/microbiological synergism was observed for
25 the As(III) concentration range of 0.2-0.5 mM with 0.1% birnessite, wherein no net Mn loss from birnessite
26 was noticed for complete As(III) oxidation. The continuous column test was run for 40 days at a HRT
27 (hydraulic retention time) of 3 hours by feeding a 0.2 mM As(III) solution. The As(III) oxidation efficiency
28 of > 98% was consistently achieved while strictly controlling the Mn²⁺ dissolution throughout the test period.
29 This study concluded that by taking advantage of a robust microbial Mn-oxidizing activity, the use of
30 “bioactive” birnessite realizes self-sustainable oxidation of As(III), without necessitating additional feed of
31 oxidant birnessite, Mn²⁺ ions or organics.

32 Introduction

33 Mn-oxides are known as naturally occurring oxidizing agents involved in a variety of redox
34 reactions of organic/inorganic species and compounds in the environment. Natural Mn-oxides found in
35 circumneutral pH environments are often poorly crystalline and considered to be of a biological origin
36 (primarily phylломanganate most similar to δ -MnO₂ or acid birnessite; Tebo et al., 2004). Microbiological
37 Mn²⁺ oxidation also follows the stoichiometry of chemical reaction represented as [Eq. 1], which is typically
38 catalyzed by multicopper oxidase enzymes of Mn-oxidizing bacteria via two sequential one-electron
39 transfer (Mn²⁺ → Mn(III) → Mn(IV); Tebo et al., 2004):



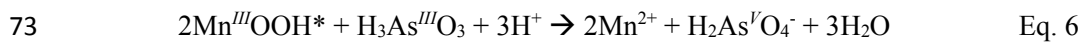
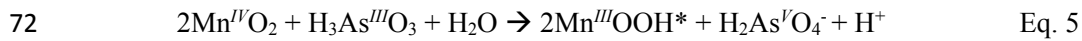
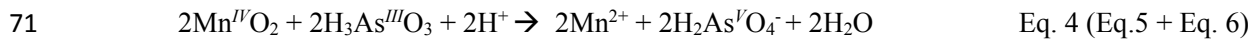
41
42 Abiotic Mn²⁺ oxidation was also reported to occur in a two-step process, in which Mn^{III}-
43 oxides/oxyhydroxides (such as hausmannite (Mn^{II, III}₃O₄) and feitknechtite (β-Mn^{III}OOH)) are initially
44 formed, followed by slower disproportionation reactions, eventually forming Mn^{IV}O₂ (rate-limiting step
45 favoring pH <9; [Eq. 2]). Whilst Mn^{III} is effectively stabilized by the comproportionation of Mn²⁺ and Mn^{IV}
46 in alkaline conditions (Murray et al., 1985; Tebo et al., 2004; Takashima et al., 2011; [Eq. 3]).



49
50
51
52 Regardless of the origin (biotic or abiotic) of Mn-oxides, geochemical cycling of a variety of metals
53 on the Earth's crust is also affected by the action of such naturally occurring oxidants (Fendorf and Zasoski,
54 1992; Manceau et al., 1997). The involvement of Mn-oxides in the oxidation of As(III) gives especially a
55 critical indication that can dictate the fate of this highly toxic metal in the environment.

56
57 Arsenic contamination in groundwater and other aquatic systems is widely reported across the
58 world, with its concentration ranging up to around 50 mg/L As (Singh et al., 2015). Inorganic As species
59 are solubilized typically in the form of arsenite (H₃As^{III}O₃) or arsenate (H₃As^VO₄), with the former more
60 toxic and soluble. The treatment of As(III)-polluted waters thus often employs the oxidation step of As(III)
61 to less toxic/soluble As(V) form. A variety of methodologies proposed for As(III) oxidation range from the
62 use of conventional chemical oxidants (i.e., Cl₂, O₃, H₂O₂) to photochemical/photocatalytic and biological
63 oxidation (Sorlini et al., 2010; Singh et al., 2015; Okibe et al., 2014; Tanaka and Okibe, 2018; Okibe and
64 Fukano, 2019). The use of Mn-oxides is also one of the approaches studied for the purpose of As(III)
65 oxidation [Eq. 4], and it is known that their reactivity can vary based on their mineralogy (Oscarson et al.,
66 1983). A number of studies reported the As(III) oxidizing ability of phylломanganates. However, it is well
67 documented that the reactivity of phylломanganates declines over time owing to secondary mineral

68 passivation (Scott et al., 1995; Manning et al., 2002; Tournassat et al., 2002). Mn^{III}-intermediates
69 (Mn^{III}OOH*) were detected during the As(III) oxidation to passivate the surface of phyllomanganates ([Eq.
70 5], [Eq. 6]; Nesbitt et al., 1998; Lafferty et al., 2010a; 2010b]:



74

75 It was also suggested that most of the resultant As(V) anions produced by [Eq. 4] are released into
76 solution, while some may remain on the mineral surface by adsorption, thus contributing to the passivation
77 of phyllomanganates. Formation of Mn-As precipitates was also suggested to become a cause of the mineral
78 passivation under certain conditions (Manning et al., 2002; Tournassat et al., 2002).

79 In order to overcome the above passivation problems and to maintain the prolonged oxidant
80 reactivity, the introduction of microbial Mn-oxidizing ability in this reaction may represent one of the
81 solutions. Watanabe et al. (2013) utilized fungal biogenic manganese oxides (BMOs) in a batch study and
82 underlined the importance of its self-regeneration to maintain the As(III) oxidation reaction. Katsoyiannis
83 et al. (2004) suggested the effectiveness of indigenous Mn- and Fe-oxidizing bacterial consortium for the
84 oxidative removal of As(III) from groundwater contaminated with low-level As, Mn and Fe ions. The
85 authors reported the occurrence of the following sequential reactions in the continuous column treatment
86 system: (i) microbial oxidation of Mn²⁺ to Mn(IV) and Fe²⁺ to Fe³⁺, (ii) microbial oxidation of As(III) to
87 As(V), (iii) precipitation of Mn^{IV}-oxides, (iv) abiotic oxidation of As(III) by Mn^{IV}-oxides, and (e) As(V)
88 adsorption on Mn^{IV}-oxides.

89 Theoretically, if the reductively-dissolved Mn²⁺ after As(III) oxidation (according to [Eq. 6]) could
90 be simultaneously re-oxidized back to Mn-oxides by Mn-oxidizing bacteria based on [Eq. 1], the As(III)
91 oxidation reaction would continue sustainably without consuming originally-provided oxidant (i.e., without
92 necessitating additional Mn²⁺ inflow). To our knowledge, such a self-sustainable (free of Mn²⁺ inflow),
93 continuous column process for the As(III) oxidation using biogenic birnessite is yet unknown. Therefore,
94 this study aimed to develop a new As(III)-oxidation treatment process targeting relatively high-level As
95 contamination in groundwater (i.e., dozens of ppm), by combining the robust bacterial Mn-oxidizing ability
96 for continuous regeneration of the oxidant birnessite.

97 2. Materials and Methods

98 2.1 Microorganism

99 Mn-oxidizing bacterium, *Pseudomonas* sp. strain SK3 isolated from a metal-refinery wastewater
100 treatment system (Kitjanukit et al. 2019) was routinely sub-cultured and maintained in half-strength
101 lysogeny broth (LB) medium (0.5% (w/v) NaCl; 0.5% (w/v) tryptone; 0.25% (w/v) yeast extract) in
102 Erlenmeyer flasks (shaken at 120 rpm, 25°C). Cells were pre-grown overnight, collected and washed with
103 0.8% (w/v) NaCl solution prior to use in the following Mn oxidation tests.

104

105 2.2 Preparation of biogenic birnessite

106 *Pseudomonas* sp. SK3 was inoculated in 1 L of peptone–yeast extract–glucose-1 (PYG-1) medium
107 (0.025% peptone; 0.025% yeast extract; 1 mM glucose; 2.02 mM MgSO₄•7H₂O; 0.068 mM CaCl₂•2H₂O;
108 4.5 g/L PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)); pH 7.0 with NaOH) containing 100 mg/L Mn²⁺
109 (as MnSO₄) and 3 μM Cu²⁺ (as CuCl₂) (in 2 L Erlenmeyer flasks). After incubation (shaken at 120 rpm,
110 25°C) for 96 hours, black birnessite precipitates were collected, washed with 0.8% NaCl solution and then
111 freeze-dried overnight prior to use in section 2.3.

112

113 2.3 As(III)-oxidation test (cycle flask batch test)

114 Biogenic birnessite (prepared in section 2.2) was added to a pulp density of 0.1% (w/v) into 100
115 mL Erlenmeyer flasks containing 50 mL of PYG-2 medium (0.01% peptone; 0.01% yeast extract; 1 mM
116 glucose; 2.02 mM MgSO₄•7H₂O; 0.068 mM CaCl₂•2H₂O; 4.5 g/L; pH 7.0 with NaOH) containing 3 μM
117 Cu²⁺. The initial As(III) concentration was set to 0.2 mM (added as NaAsO₂). In order to evaluate the effect
118 of active Mn-oxidizing cells in the system, tests were set up with and without pre-grown *Pseudomonas* sp.
119 SK3 cells (10⁹ cells/mL). Birnessite-free controls containing only SK3 cells (10⁹ cells/mL) were also set-
120 up in parallel. The flasks were incubated shaken at 120 rpm, 25°C for 24 hours, followed by centrifugation
121 to replace the supernatant with the same fresh medium. This procedure was repeated three times. All tests
122 were conducted in duplicate flasks. Samples were withdrawn regularly to monitor pH, Eh (vs. SHE),
123 planktonic cell density, As(III) concentration (molybdenum blue method; Blomqvist et al., 1993; Murphy
124 and Riley, 1962) and total Mn and As concentrations (ICP-OES; Optima8300, Perkin Elmer). Precipitates
125 were collected after the 1st- and 3rd-cycles (at 24 and 72 hours) and freeze-dried overnight for X-ray
126 diffraction (XRD) (Ultima IV, Rigaku; Cu Kα 40 mA, 40 kV) and X-ray absorption near-edge structure
127 (XANES) analyses.

128 Additionally, the effect of different initial As(III) concentrations (0.2-0.7 mM) was compared in a
129 single batch test to evaluate the robustness of this microbially-catalyzed coupling reaction at higher As
130 toxicity levels.

131

132 **2.4 X-ray absorption near-edge structure (XANES)**

133 Biogenic birnessite samples (collected in section 2.3) were quantitatively mixed with boron nitride
134 and pressed into a tablet. The Mn K-edge spectra were collected (transmission mode; 6200-8500 eV) at
135 SAGA-LS (1.4 GeV, 75.6 m; Kyushu University Beam Line 06). Average Mn oxidation state (AOS) of
136 biogenic birnessite was calculated based on the linear combination fitting of Mn K-edge XANES spectra
137 (6200-6600 eV) using the Athena program (Demeter version 0.9.24).

138

139 **2.5 Comparison of As(III)-oxidizing reactivity of Mn^{IV}O₂ and Mn^{III}₂O₃ (chemical reagents)**

140 MnO₂ (Wako; 138-09675) or Mn₂O₃ (Kojundo chemical laboratory; 215558) was added to a pulp
141 density of 0.15 % into 100 mL Erlenmeyer flasks containing 50 mL of PIPES solution (4.5 g/L; pH 7.0
142 with NaOH) containing 1.8 mM As(III). The flasks were incubated shaken at 120 rpm, 25°C. Samples were
143 withdrawn to monitor As(III) and total Mn concentrations.

144

145 **2.6 Continuous As(III)-oxidation column test**

146 Natural zeolite from Shimane, Japan (No.5, 0.5-1.5 mm, Shinsei Corporation; 68% SiO₂, 12%
147 Al₂O₃, 2.5% Na₂O, 1.9% K₂O, 1.7% CaO; specific surface area 246 m²/g) was used as supporting material
148 for biogenic birnessite and Mn-oxidizing bacterial cells ("Bio-zeolite"). Bio-zeolite was prepared as
149 follows: *Pseudomonas* sp. SK3 cells (at a density of 10⁹ cells/mL) and 50 g zeolite particles were added
150 into 1 L of PYG-1 medium (pH 7.0 with NaOH; 2 L Erlenmeyer flasks) containing 100 mg/L Mn²⁺ and 3
151 μM Cu²⁺. After 96-hour incubation (at 120 rpm, 25°C), the supernatant was discarded by decantation and
152 replaced with the same fresh medium. This was repeated three times and the products were packed into the
153 column. The first column run was carried out while altering the HRT from 12 hours to 2 hours to look for
154 the optimal speed. For the second column run, the HRT was fixed at 3 hours. Synthetic As(III)-polluted
155 water used as feedwater contained 2.02 mM MgSO₄·7H₂O, 0.068 mM CaCl₂·2H₂O, 4.5 g/L PIPES, 3 μM
156 Cu²⁺ and 0.2 or 0.4 mM As(III); pH 7.0 with NaOH). **PIPES was removed from the feed water on day 27**
157 **to eliminate any artificial buffering effect.** Liquid samples were regularly withdrawn from the inlet/outlet
158 of the column to monitor pH, Eh (vs. SHE) and the concentration of As(III), total Mn and total As.

159 **3. Results and discussion**

160 **3.1 Mn-oxidizing bacteria simultaneously regenerate birnessite during the chemical As(III) oxidation**
161 **by birnessite**

162 At first, in order to clarify the capability of real-time Mn oxidation by *Pseudomonas* sp. SK3 during
163 the chemical oxidation of highly toxic As(III) by birnessite, tests were set up with or without the presence
164 of active cells. Freeze-dried biogenic birnessite was added only once at 0.1% before initiating the 1st-cycle,
165 and neither additional birnessite nor any form of Mn was supplemented thereafter. The absence of microbial
166 As(III)-oxidizing ability was confirmed as a premise (Fig. 1a, b).

167 According to [Eq. 4], the initial dose of 0.1% biogenic birnessite is calculated to be over 20-folds
168 of the stoichiometric amount required for complete oxidation of 0.2 mM As(III) (approx. 86% of Mn in
169 biogenic birnessite used in this test exists as Mn(IV)). Under the abiotic condition, however, this excess
170 birnessite oxidized only 63% of As(III) during the 1st-cycle (Fig. 1a), accompanied by a release of 0.4 mM
171 Mn^{2+} (9% of Mn lost from the initially added biogenic birnessite). Since fresh biogenic birnessite contain
172 some adsorbed Mn^{2+} ions (Santisak et al. 2019), the released Mn^{2+} in the 1st-cycle is comprised of some
173 desorbed Mn^{2+} ions plus reductively-dissolved Mn^{2+} ions (Fig. 1c, d). The 1st-cycle in the abiotic condition
174 was followed by increasingly weaker As(III) oxidation in the 2nd- and 3rd-cycles (Fig. 1a). Mn^{2+}
175 dissolution continued and the amount of Mn lost from the initial birnessite dose became 19% by the end of
176 the 3rd cycle (Fig. 1d).

177 In contrast, the presence of active Mn-oxidizing cells led to complete oxidation of 0.2 mM As(III)
178 within 24 hours in the 1st-cycle (Fig. 1a). Despite that the net amount of birnessite was constant throughout
179 the cycle test (unless dissolved), the As(III) oxidation speed in the biotic condition became even higher in
180 the following 2nd- and 3rd-cycles (As(III) oxidation completed within 6 hours; Fig. 1a) and Mn^{2+}
181 dissolution was negligible (Fig. 1c, d).

182 Under conditions where As(III) oxidation was proceeded (birnessite \pm cells), a small portion of
183 the total soluble As was immobilized in each cycle, especially at later cycles (Fig. 1b). This is likely due to
184 the adsorption of the resultant As(V) on the birnessite surface, which was reported to occur at crystallite
185 edges and interlayer domains (Manning et al., 2002). Formation of Mn-As precipitates was unlikely in this
186 study since ion concentrations were far below its K_{sp} (Schacht and Ginder-Vogel, 2018; Tournassat et al.,
187 2002; Sadiq, 1997). The reason for a slightly increased As(V) adsorption at later cycles (Fig. 1b) may be
188 attributed to the As(III) oxidation reaction causing a surface alteration to create fresh reaction sites for
189 As(V) on birnessite surfaces (Manning et al., 2002). A slightly less As(V) adsorption in 1st- and 2nd-cycles
190 in the birnessite + cells condition than in the cell-free counterpart may have been caused by any organic
191 passivation on the mineral surface; however, this trend was reversed in the 3rd-cycle, possibly because

192 continuously extensive As(III) oxidation in the biotic system more readily created As(V) adsorption sites
193 (Manning et al., 2002). Although Parikh et al. (2010) reported the negative influence of bacteria/biopolymer
194 coatings on the initial As(III) oxidation kinetics by δ -MnO₂, this study showed that the advantage of
195 microbial Mn-oxidizing activity was far greater than the possible passivation effect caused by their biomass.
196

197 **3.2 The average oxidation state (AOS) of birnessite increases through its dissolution-recrystallization** 198 **process mediated by Mn-oxidizing bacteria**

199 The reasons for the increased As(III) oxidation efficiency during the cycle batch test in biotic
200 conditions (section 3.1) were investigated by XANES and XRD analyses. The AOS of biogenic birnessite
201 before starting the cycle As(III) oxidation test (at 0 hours) was 3.80 (Mn^{IV} 86.1%; Mn^{III} 7.4%; Mn^{II} 6.5%),
202 which then gradually decreased down to 3.69 (Mn^{IV} 78.2%; Mn^{III} 12.1%; Mn^{II} 9.7%) after 3 cycles in the
203 abiotic condition (Fig. 2). The trend was reversed in the biotic condition and the AOS increased up to 3.94
204 (Mn^{IV} 95.2%; Mn^{III} 3.6%; Mn^{II} 1.2%) after 3 cycles (Fig. 2).

205 The XRD results indicated no evidence of improved birnessite crystallinity during the cycle test,
206 and no apparent difference in the XRD peaks was found between the abiotic and biotic conditions
207 (Supplemental Fig. 1). Another possible reason for the improved As(III) oxidation rate during the cycle test
208 in the biotic condition can be attributed to the cell density in the system: Whilst the planktonic cell density
209 was mostly stable (at 6-8 x 10⁸ cell/mL in all cycles), sessile/trapped cells on the birnessite surface may
210 have increased during the microbial birnessite regeneration process, eventually improving the As(III)
211 oxidation rate.

212 Decreased As(III) oxidation rate (Fig. 1a) accompanied by the lowered Mn AOS (Fig. 2) in the
213 abiotic condition can be explained by passivation of the birnessite surface with Mn^{III}-products formed
214 during As(III) oxidation by birnessite [Eq. 5, 6] as well as through comproportionation reaction [Eq. 3].
215 This observation was not evidenced by the XRD analysis (Supplemental Fig. 1), since such Mn^{III}-
216 intermediates are likely poorly crystalline. A separate test compared the reactivity of Mn(VI) and Mn(III)
217 towards As(III) oxidation (by using reagent-grade crystalline Mn^{III}₂O₃ and α -Mn^{IV}O₂). As shown in Fig. 3,
218 As(III) oxidation by Mn(III) was much weaker than that by Mn(IV), supporting the correlation between the
219 decreased As(III) oxidation rate and the lowered Mn AOS.

220 The results from 3.1 and 3.2 confirmed that the combination of chemical As(III) oxidation by
221 birnessite and microbial birnessite regeneration by Mn-oxidizing bacteria enables self-sustainable As(III)
222 oxidation reaction. This effective synergism was seen for the initial As(III) concentration range of 0.2-0.5
223 mM (Fig. 4): Within this range, the molar ratio of [Mn²⁺ released]/[As(III) oxidized] (Mn/As ratio) at the
224 very beginning was 1-2 (Fig. 4c), reflecting the spontaneous chemical As(III) oxidation according to Eq. 4
225 together with some desorbed Mn²⁺ ions from the birnessite structure. The Mn/As ratio then quickly shifted

226 to negative owing to microbial birnessite regeneration, with its speed affected by the toxic As(III) level
227 (Fig. 4c). There was no net Mn loss from birnessite for completion of As(III) oxidation (Fig. 4b). On the
228 other hand, at the highest initial As(III) concentration of 0.7 mM, a significant amount of Mn^{2+} was released
229 (Fig. 4b) due to deactivation of Mn-oxidizing ability by elevated As(III) toxicity and the As(III) oxidation
230 was not completed (Fig. 4a).

231

232 3.3 Continuous self-regenerative bio-zeolite column can process As(III) oxidation at > 98% efficiency

233 Following the above batch tests, the continuous As(III) oxidation column was set up, as shown in
234 Fig. 5. Firstly, a suitable HRT range was searched at 0.2 mM As(III). As shown in Fig. 6a, high As(III)
235 oxidation efficiencies (94-100%) were observed at HRT from 12 hours to 4 hours. However, soon after the
236 HRT shortened to 2 hours, Mn^{2+} started to dissolve from bio-zeolite and the column efficiency progressively
237 deteriorated; dissolution of only a few percent of Mn from bio-zeolite was shown to seriously worsen the
238 column performance (Fig. 6a). This observation reconfirmed the importance of active Mn-oxidizing
239 bacteria in the system to strictly control the regeneration of birnessite in the column. Except for temporal
240 sorption of a slight amount of As(V) at the beginning, no clear evidence of As(V) sorption was seen, since
241 total As concentrations at the inlet and outlet were nearly identical throughout the column run (Fig. 6b).
242 This observation suggests that birnessite in the column was also free from major passivation via As(V)
243 sorption.

244 The next column test was conducted at the fixed HRT of 3 hours (Fig. 7) at 0.2 mM As(III) for
245 40 days. Although *Pseudomonas* sp. SK3 used in this test is a heterotrophic Mn-oxidizer (Kitjanukit et al.
246 2019), the column test was run without any additional organic sources. For the 40-days-long test period,
247 high As(III) oxidation efficiencies (> 98%) were maintained (Fig. 7a) with strictly controlled Mn^{2+}
248 dissolution (< 0.006 mM; 0.33 mg/L; Fig. 7b) through robust birnessite regeneration by Mn-oxidizing cells.
249 Although there was some pH fluctuation observed after removing the buffering agent PIPES on day 27 (Fig.
250 7b), the trends of As(III) oxidation and Mn^{2+} dissolution were unaffected (Fig. 7). It can be postulated that
251 the growth of heterotrophic Mn-oxidizing cells relied upon organic substances deriving from their own cell
252 lysates/exudates. This self-sustainable feeding cycle may have proceeded via toxicity of As(III), which
253 possibly accelerated cell regenerations in turn. It is, however, possible that some organics needs to be
254 externally injected to the system at some point, if the column was to run for a much longer period.

255 In abiotic stirred-flow reactor studies by Lafferty et al. (2010a, b), oxidation of 0.1 mM As(III) by
256 δ - MnO_2 initially proceeded rapidly but slowed down considerably within several hours, due to passivation
257 of δ - MnO_2 by Mn^{III} -precipitates (as a result of comproportionations of sorbed Mn^{2+} and Mn(IV) [Eq. 3],
258 rather than Mn(IV) reduction by As(III) [Eq. 4]) as well as by sorption of Mn^{2+} and As(V).

259 On the other hand, our column reactor packed with “bioactive” birnessite-coated zeolite maintained
260 its high reactivity for over 40 days. The results obtained in the cycle batch test (section 3.1, 3.2) explain the
261 underlying mechanism in the column; robust real-time regeneration of birnessite by active Mn-oxidizing
262 cells strictly controlled the Mn²⁺ dissolution and avoided accumulation of Mn^{III}-precipitates. The AOS of
263 birnessite was likely continuously increased during the column run. There might have been some inhibitory
264 effect of As(V) adsorption onto the birnessite surface due to the presence of cells, as was reported in a study
265 by Jones et al. (2012), wherein the presence of As(III)-oxidizing soil bacteria synergistically improved the
266 As(III) oxidation rate by δ -MnO₂, but lowered the amount of As(V) adsorption on the mineral surface.
267 Katsoyiannis et al. (2004) reported the improved As(III) oxidation rate by synergism between Mn-oxide
268 and indigenous Mn- and Fe-oxidizing bacteria for the treatment of groundwater containing both low-level
269 As(III) (35 μ g/L) and Mn²⁺ (0.6 mg/L). This study demonstrated that by employing a robust Mn-oxidizing
270 species, bioactive birnessite is capable of continuous, self-sustainable oxidation of high-level As(III)
271 contaminants in groundwater without even necessitating additional feed of oxidant, Mn²⁺, or organics, via
272 simultaneous regeneration of the oxidant birnessite while avoiding mineral passivation.

273 **4. Conclusions**

- 274 ● Highly toxic As(III) was chemically oxidized to less toxic/soluble As(V) by biogenic birnessite, while
275 the reaction was incomplete and the reactivity rapidly deteriorated during the cycle batch test.
- 276 ● The synergism between chemical As(III) oxidation by birnessite and simultaneous birnessite
277 regeneration by Mn-oxidizing *Pseudomonas* SK3 cells enabled complete oxidation of As(III), and the
278 oxidation rate increased during the cycle batch test.
- 279 ● The XANES analysis suggested that passivation of the birnessite surface with Mn^{III}-mineral caused
280 the declined reactivity in abiotic conditions (birnessite AOS decreased from 3.8 to 3.69 during the
281 cycle batch test), while the improved As(III) oxidation rate in the biotic counterpart corresponded to
282 the increased birnessite AOS from 3.8 to 3.94.
- 283 ● The above chemical/microbial synergism was effective for the As(III) concentration range of 0.2-0.5
284 mM in the presence of 0.1% birnessite, where no net Mn loss from birnessite was noticed for complete
285 As(III) oxidation.
- 286 ● The continuous column test showed the As(III) oxidation efficiency of > 98% throughout the 40 days
287 experiment at the HRT of 3 hours using 0.2 mM As(III) solution, wherein Mn²⁺ dissolution was
288 negligible.
- 289 ● By employing a robust Mn-oxidizing bacterial species, bioactive birnessite enabled continuous, self-
290 sustainable oxidation of As(III) without necessitating additional feed of oxidant, Mn²⁺, or organics, via
291 simultaneous regeneration of birnessite while avoiding the mineral passivation with Mn^{III}-precipitates
292 or adsorbed As(V) ions.
- 293

294 **Acknowledgment**

295 The XAFS experiment was performed at the SAGA Light Source (Kyushu University Beam Line; BL06,
296 No.2019IHK007). This work was partly supported by Japan Oil, Gas and Metals National Corporation
297 (JOGMEC).

298

299 **Reference**

300 Blomqvist, S., Hjellström, K., Sjösten, A., 1993. Interference from arsenate, fluoride and silicate when
301 determining phosphate in water by the phosphoantimonyl molybdenum blue method. *Int. J. Environ. Anal.*
302 *Chem.* 54, 31-43. <https://doi:10.1080/03067319308044425>.

303

304 Fendorf, S.E., Zasoski, R.J., 1992. Chromium(III) oxidation by δ -MnO₂. 1. Characterization. *Environ. Sci.*
305 *Technol.* 26, 79-85. <https://doi.org/10.1021/es00025a006>.

306

307 Jones, L.C., Lafferty, B.J., Sparks, D.L., 2012. Additive and competitive effects of bacteria and Mn oxides
308 on arsenite oxidation kinetics. *Environ. Sci. Technol.* 46, 6548-6555. <https://doi.org/10.1021/es204252f>.

309

310 Katsoyiannis, I.A., Zouboulis, A.I., Jekel, M., 2004. Kinetics of bacterial As(III) oxidation and subsequent
311 As(V) removal by sorption onto biogenic manganese oxides during groundwater treatment. *Ind. Eng. Chem.*
312 *Res.* 43, 486-493. <https://doi.org/10.1021/ie030525a>.

313

314 Kitjanukit, S., Takamatsu, K., Okibe, N., 2019. Natural attenuation of Mn(II) in metal refinery wastewater:
315 microbial community structure analysis and isolation of a new Mn(II)-oxidizing bacterium *Pseudomonas*
316 *sp.* SK3. *Water* 11, 507. <https://doi.org/10.3390/w11030507>.

317

318 Lafferty, B.J., Ginder-Vogel, M., Sparks, D.L., 2010a. Arsenite oxidation by a poorly crystalline
319 manganese-oxide 1. Stirred-flow experiments. *Environ. Sci. Technol.* 44, 8460-8466.
320 <https://doi.org/10.1021/es102013p>.

321

322 Lafferty, B.J., Ginder-Vogel, M., Zhu, M., Livi, K.J.T., Sparks, D.L., 2010b. Arsenite oxidation by a poorly
323 crystalline manganese-oxide. 2. Results from X-ray absorption spectroscopy and X-ray diffraction. *Environ.*
324 *Sci. Technol.* 44, 8467-8472. <https://doi.org/10.1021/es102016c>.

325

326 Manceau, A., Silvester, E., Bartoli, C., Lanson, B., Drits Victor, A., 1997. Structural mechanism of Co^{2+}
327 oxidation by the phylломanganate buserite. *Am. Mineral.* 82, 1150–1175. [https://doi.org/10.2138/am-1997-](https://doi.org/10.2138/am-1997-11-1213)
328 11-1213.

329

330 Manning, B.A., Fendorf, S.E., Bostick, B., Suarez, D.L., 2002. Arsenic(III) oxidation and Arsenic(V)
331 adsorption reactions on synthetic birnessite. *Environ. Sci. Technol.* 36, 976-981.
332 <https://doi.org/10.1021/es0110170>.

333

334 Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in
335 natural waters. *Anal. Chim. Acta* 27, 31-36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5).

336

337 Murray, J.W., Dillard, J.G., Giovanoli, R., Moers, H., Stumm, W., 1985. Oxidation of Mn(II)-initial
338 mineralogy, oxidation-state and aging. *Geochim. Cosmochim. Acta* 49, 463-470.
339 [https://doi.org/10.1016/0016-7037\(85\)90038-9](https://doi.org/10.1016/0016-7037(85)90038-9).

340

341 Nesbitt, H.W., Canning, G.W., Bancroft, G.M., 1998. XPS study of reductive dissolution of 7Å-birnessite
342 by H_3AsO_3 , with constraints on reaction mechanism. *Geochim. Cosmochim. Acta* 62, 2097-2110.
343 [https://doi.org/10.1016/S0016-7037\(98\)00146-X](https://doi.org/10.1016/S0016-7037(98)00146-X).

344

345 Okibe, N., Fukano, Y., 2019. Bioremediation of highly toxic arsenic via carbon-fiber assisted indirect
346 As(III) oxidation by moderately thermophilic, acidophilic Fe-oxidizing bacteria. *Biotech. Lett.* 41, 1403–
347 1413. <https://doi.org/10.1007/s10529-019-02746-7>.

348

349 Okibe, N., Koga, M., Morishita, S., Tanaka, M., Heguri, S., Asano, S., Sasaki, K., Hirajima, T., 2014.
350 Microbial formation of crystalline scorodite for treatment of As(III)-bearing copper refinery process
351 solution using *Acidianus brierleyi*. *Hydrometallurgy* 143, 34-41.
352 <https://doi.org/10.1016/j.hydromet.2014.01.008>.

353

354 Oscarson, D.W., Huang, P.M., Liaw, W.K., Hammer, U.T., 1983. Kinetics of oxidation of arsenite by
355 various manganese dioxides. *Soil Sci. Soc. Am. J.* 47, 644-648.
356 <https://doi.org/10.2136/sssaj1983.03615995004700040007x>.

357

358 Parikh, S.J., Lafferty, B.J., Meade, T.G., Sparks, D.L., 2010. Evaluating environmental influences on AsIII
359 oxidation kinetics by a poorly crystalline Mn-oxide. *Environ. Sci. Technol.* 44, 3772-3778.
360 <https://doi.org/10.1021/es903408g>.

361

362 Sadiq, M., 1997. Arsenic chemistry in soils: An overview of thermodynamic predictions and field
363 observations. *Water Air Soil Pollut.* 93, 117-136. <https://doi.org/10.1007/bf02404751>.

364

365 Schacht, L., Ginder-Vogel, M., 2018. Arsenite depletion by manganese oxides: A case study on the
366 limitations of observed first order rate constants. *Soil Syst.* 2, 39.
367 <https://doi.org/10.3390/soilsystems2030039>.

368

369 Scott, M.J., Morgan, J.J., 1995. Reactions at oxide surfaces. 1. Oxidation of As(III) by synthetic birnessite.
370 *Environ. Sci. Technol.* 29, 1898-1905. <https://doi.org/10.1021/es00008a006>.

371

372 Singh, R., Singh, S., Parihar, P., Singh, V.P., Prasad, S.M., 2015. Arsenic contamination, consequences and
373 remediation techniques: A review. *Ecotoxicol. Environ. Saf.* 112, 247-270.
374 <https://doi.org/10.1016/j.ecoenv.2014.10.009>.

375

376 Sorlini, S., Gialdini, F., 2010. Conventional oxidation treatments for the removal of arsenic with chlorine
377 dioxide, hypochlorite, potassium permanganate and monochloramine. *Water Res.* 44, 5653-5659.
378 <https://doi.org/10.1016/j.watres.2010.06.032>.

379

380 Takashima, T., Hashimoto, K., Nakamura, R., 2012. Mechanisms of pH-dependent activity for water
381 oxidation to molecular oxygen by MnO₂ electrocatalysts. J. Am. Chem. Soc. 134, 1519-1527.
382 <https://doi.org/10.1021/ja206511w>.

383

384 Tanaka, M., Okibe, N., 2018. Factors to enable crystallization of environmentally stable bioscorodite from
385 dilute As(III)-contaminated waters. Minerals 8, 23. <https://doi.org/10.3390/min8010023>.

386

387 Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J., Parker, D., Verity, R., Webb, S.M., 2004.
388 Biogenic manganese oxides: properties and mechanisms of formation. Annu. Rev. Earth Planet. Sci. 32,
389 287-328. <https://doi.org/10.1146/annurev.earth.32.101802.120213>.

390

391 Tournassat, C., Charlet, L., Bosbach, D., Manceau, A., 2002. Arsenic(III) oxidation by birnessite and
392 precipitation of manganese(II) arsenate. Environ. Sci. Technol. 36, 493-500.
393 <https://doi.org/10.1021/es0109500>.

394

395 Watanabe, J., Tani, Y., Chang, J., Miyata, N., Naitou, H., Seyama, H., 2013. As(III) oxidation kinetics of
396 biogenic manganese oxides formed by *Acremonium strictum* strain KR21-2. Chem. Geol. 347, 227-232.
397 <https://doi.org/10.1016/j.chemgeo.2013.03.012>.

398 **Figure legends**

399 Fig. 1. Oxidation of As(III) to As(V) coupled with the reduction of biogenic birnessite to Mn^{2+} in the cycle
400 batch test: As(III) concentrations (a), total soluble As concentrations (b), total soluble Mn concentrations
401 (c) and Mn loss from birnessite (d) are shown for each cycle. Birnessite was added only once before the
402 1st-cycle in all cases (at a pulp density of 0.1%). Likewise, active Mn-oxidizing *Pseudomonas* sp. SK3 cells
403 were added only once before the 1st-cycle (at 10^9 cells/mL) (●). Cell-free controls containing only
404 birnessite (×), as well as birnessite-free controls containing only SK3 cells (○) were also prepared in parallel.

405

406 Fig. 2. Transition of the Mn species and AOS (average oxidation state) of birnessite during the cycle batch
407 As(III) oxidation test with or without the presence of Mn-oxidizing *Pseudomonas* sp. SK3 cells (shown in
408 Fig. 1): The ratio of Mn^{2+} (white), Mn(III) (grey) and Mn(IV) (black) (left) were calculated from the linear
409 combination fitting result (dotted lines) of Mn K-edge XANES spectra (solid lines) (right). As Mn standards,
410 $Mn^{II}SO_4$, $Mn^{III}_2O_3$ and δ - $Mn^{IV}O_2$ were used.

411

412 Fig. 3. Comparison of As(III)-oxidizing reactivity between α - $Mn^{IV}O_2$ (●) and $Mn^{III}_2O_3$ (▲) (both are
413 chemical reagents).

414

415 Fig. 4. The effect of initial As(III) concentration on chemical/microbial synergistic cycle between As(III)
416 oxidation by birnessite and birnessite regeneration: The initial As(III) concentration was set to 0.2 mM (●),
417 0.4 mM (○), 0.5 mM (▲) or 0.7 mM (△). Changes in As(III) concentrations (a), total soluble Mn
418 concentrations (b) and the molar ratio of the released Mn^{2+} ions to oxidized As(III) ions (c) are shown. Grey
419 lines in (c) were hand-drawn to show the approximate trend in each condition.

420

421 Fig. 5. Schematic diagram of the column reactor setup: Natural zeolite particles supporting biogenic
422 birnessite and active Mn-oxidizing *Pseudomonas* sp. SK3 cells (“bio-zeolite”) were packed in the acryl
423 column. The As(III) concentration in feed water was set to 0.2 mM. PIPES was removed from the feed
424 water from day 27 on.

425

426 Fig. 6. Continuous As(III) oxidation in the bio-zeolite column reactor at different HRTs (12, 6, 4 and 2
427 hours): (a) As(III) oxidation efficiency (●) vs. Mn lost from the bio-zeolite (○). (b) Total soluble As
428 concentrations at the inlet (△) or outlet (▲) of the column.

429

430 Fig. 7. Continuous As(III) oxidation in the bio-zeolite column reactor at the fixed HRT of 3 hours. (a)

431 As(III) oxidation efficiency (\times) and As(III) concentrations at the inlet (Δ) or outlet (\blacktriangle). (b) Mn dissolved

432 from birnessite (\blacktriangle) and pH at the inlet (\square) or outlet (\blacksquare).

433

434

435 Supplemental Fig.1. XRD diffraction patterns of biogenic birnessite before (0 hours) and after (72 hours)

436 the cycle batch As(III) oxidation test, with or without the presence of Mn-oxidizing *Pseudomonas* sp. SK3

437 cells (shown in Fig. 1). ●, birnessite (JCDD 43-1456).