

1 Speciation of arsenic in a thermoacidophilic iron-oxidizing archaeon, *Acidianus*
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4 *brierleyi*, and its culture medium by inductively coupled plasma-optical
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7 emission spectroscopy combined with flow injection pretreatment using an
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10 anion-exchange mini-column
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ABSTRACT

The thermoacidophilic iron-oxidizing archaeon *Acidianus brierleyi* is a microorganism that could be useful in the removal of inorganic As from wastewater, because it simultaneously oxidizes As(III) and Fe(II) to As(V) and Fe(III) in an acidic culture medium, resulting in the immobilization of As(V) as FeAsO₄. To investigate the oxidation mechanism, speciation of the As species in both the cells and its culture media is an important issue. Here we describe the successive determination of As(III), As(V), and total As in *A. brierleyi* and its culture medium via a facile method based on inductively coupled plasma-optical emission spectroscopy (ICP-OES) with a flow injection pretreatment system using a mini-column packed with an anion-exchange resin. The flow-injection pretreatment system consisted of a syringe pump, a selection valve, and a switching valve, which were controlled by a personal computer. Sample solutions with the pH adjusted to 5 were flowed into the mini-column to retain the anionic As(V), whereas As(III) was introduced into ICP-OES with no adsorption on the mini-column due to its electrically neutral form. An acidic solution (1 M HNO₃) was then flowed into the mini-column to elute As(V) followed by ICP-OES measurement. The same sample was also subjected to ICP-OES without being passed through the mini-column in order to determine the total amounts of As(III) and As(V). The method was verified by comparing the results of the total As with the sum of As(III) and As(V). The calibration curves showed good linearity with limits of detection of 158 ppb, 86 ppb and 211 ppb for As(III), As(V) and total As, respectively. The method was successfully applicable to the determination of the As species contained in the pellets of *A. brierleyi* and their culture media. The results suggested that the oxidation of As(III) was influenced by the presence of Fe(II) in the culture medium, i.e., Fe(II) enhanced the oxidation of As(III) in *A. brierleyi*. In addition, we found that no soluble As species was contained in the cell pellets and more than 60% of the As(III) in the culture medium was oxidized by *A. brierleyi* after a 6-day incubation.

1. Introduction

Arsenic (As) is an element that is known to be toxic to humans, but the degree of toxicity depends on whether the chemical species are classified as inorganic or organic[1,2]. The inorganic species include arsenite (As(III)) and arsenate (As(V)), which are known to be more toxic than the organic species such as monomethylarsinic acid and dimethylarsinic acid. Among these species, As(III) is the most toxic, followed by As(V) and the organic varieties. Therefore, the speciation of the As species, particularly of the inorganic species, is an important issue in environmental chemistry, in earth resource engineering, and in the mining industry.

When determining the speciation of inorganic As, the simultaneous determination of three species, As(III), As(V), and total As, enhances the reliability of the results. Ordinarily, speciation is performed by the determination of two of the three As species. Several separation techniques such as high-performance liquid chromatography (HPLC) [3], ion chromatography (IC) [4,5], and capillary electrophoresis [6-8] were coupled using either inductively coupled plasma-optical emission spectroscopy (ICP-OES) or inductively coupled plasma-mass spectroscopy (ICP-MS) to achieve a sensitive determination of As(III) and As(V), whereas voltammetry was utilized for the determination of As(III) and total As, which must be determined after the chemical reduction of As(V) [9,10]. The separation of As(III) and As(V) requires an extended amount of time for analysis (approximately 10 to 30 min). Furthermore, sequential injections of biological samples may degrade the performance of the separation column for HPLC and IC since the adsorption of biological molecules such as proteins, lipids, and DNA should rapidly deteriorate the column. Compared to chromatographic methods, an automated flow injection system with solid-phase extraction has shown promise for reducing the analysis time and for solving the problem of damage to the analytical columns. For example, Jitmanee and coworkers have demonstrated a method for the speciation of As(III) and As(V) by ICP-OES coupled with a pretreatment system using two mini-columns to concentrate both As species, As(III) and As(V) [11]. This method was verified

1 when it was used to determine the content of As(III) and As(V) in natural river water.

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3 A convenient method for As speciation was important in the study of As immobilization using
4 microorganisms. Okibe and coworkers reported that the microorganism would be useful for the
5 immobilization of As species from acidic refinery wastewater via the simultaneous oxidation of
6 As(III) to As(V) followed by the formation of insoluble species consisting of Fe(III) and As(V) using
7 *Acidianus brierleyi* [12]. They demonstrated that the microorganism would be useful for the
8 removal of As from the refinery wastewater that is produced in the mining industry, although the
9 details surrounding the oxidation mechanism of *A. brierleyi* remain unclear. In their research, As
10 species in the culture media were determined via voltammetry and ICP-OES, i.e., As(III) and total As
11 were determined independently by voltammetry and ICP-OES, respectively. Therefore, a simple
12 and easy method for the determination of the As species not only in culture media but also on cells
13 would be attractive and useful in the study of the oxidation mechanism in *A. brierleyi*.
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29 In the present study, we developed a method for the speciation of As by ICP-OES coupled with a
30 flow injection pretreatment system using a single anion-exchange mini-column. The flow injection
31 system consisted of a syringe pump, a selection valve, and a switching valve, which were controlled
32 by a personal computer. Sample solutions were introduced into ICP-OES with and without passing
33 through an anion-exchange mini-column to determine As(III), As(V) and total As in succession.
34 The results obtained by the proposed method were more reliable than the other speciation methods
35 since the total concentration was also determined simultaneously and could be directly compared
36 with the determined concentrations of As(III) and As(V).
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48 The developed method is facile, high-throughput, sensitive, selective, and reliable for the
49 speciation of As and is applicable to the speciation of As in *A. brierleyi* and its culture media after
50 different incubation times. To the best of our knowledge, this is the first report concerning the
51 determination of the As species in *A. brierleyi* cells. Therefore, the method for speciation of
52 microorganisms has been successfully proven to work using *A. brierleyi* in this study. The results
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1 for the culture media were consistent with a previous report by Okibe and coworkers. In addition,
2 we found that As(III) was completely oxidized and immobilized in the microorganism after a 6-day
3 incubation.
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10 **2. Experimental**

11 *2.1. Materials*

12 All reagents were of analytical grade and were used without further purification. All
13 solutions were prepared by being dissolved in water that had been purified by means of an ultrapure
14 MILLI-Q system (Millipore, Molsheim, France). Stock solutions of As(III) and As(V) (1,000 mg
15 L⁻¹) were prepared by dissolving sodium arsenite (NaAsO₂) and disodium hydrogen arsenate
16 heptahydrate (Na₂HAsO₄·7H₂O), which were obtained from Nacalai tesque, Inc. (Kyoto, Japan). A
17 standard solution of 1,000 mg L⁻¹ Fe(III) (iron(III) nitrate in 0.1 M nitric acid), sodium acetate,
18 iron(II) sulfate heptahydrate, and ammonium iron(II) sulfate hexahydrate were purchased from Wako
19 pure chemicals (Osaka, Japan). A stock solution of Fe(II) was prepared by dissolving ammonium
20 iron(II) sulfate hexahydrate in water with the addition of a small amount of sulfuric acid (Electronic
21 grade, Mitsubishi chemicals, Tokyo, Japan). The stock solutions were diluted daily in order to
22 prepare standard solutions for constructing calibration curves. A 0.1 M sodium acetate solution was
23 used as a preconditioning solution for the mini-column packed with anion-exchange resin (Wako
24 DOWEX 1 × 4 50-100 Mesh, Wako pure chemicals, Osaka, Japan). An acetate buffer solution (pH
25 5.0) was prepared by mixing 0.1 M acetic acid (Electronic grade, Kanto chemical Co. Inc, Tokyo,
26 Japan) and 0.1 M sodium acetate solutions and the pH was monitored using a pH meter D-52
27 (HORIBA, Ltd., Kyoto, Japan). Nitric acid (electronic grade) was obtained from Mitsubishi
28 chemical (Tokyo, Japan).
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54 *2.2. Sample preparation*

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1 The microorganisms used in this study, *A. brierleyi* (DSMZ 1651), were cultured in the same
2 manner as that reported in a previous paper [12,13]. Briefly, *A. brierleyi* was cultured in a 500 mL
3 Erlenmeyer flask containing 200 mL of 9K basal salts medium (pH was adjusted to 1.5 with H₂SO₄)
4 with 500 mg L⁻¹ As(III) in either the presence or absence of 900 mg L⁻¹ Fe(II) as FeSO₄· 7H₂O. The
5 microorganisms were incubated at 70 °C with shaking at 100 rpm. The culture medium was
6 centrifuged to separate the microorganisms, which were then collected for the measurement of the As
7 species.
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10 Under these conditions, the number of cells was observed to decrease [12], which was
11 attributed to both a lack of an energy source and the toxicity of As(III) to *A. brierleyi*. However,
12 As(III) was only weakly toxic, since the *A. brierleyi* culture was still alive even after 14-days
13 incubation although a gradual decrease in the number of cells was observed [12].
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16 For the preparation of the culture medium samples, 0.25 mL of culture medium was diluted to
17 5 mL by the addition of 2.5 mL of 0.1 M acetate buffer to adjust the pH to 5.0, which resulted in a
18 500-fold diluted culture medium sample solution containing 10 mM acetate buffer. The cell pellets
19 were dissolved with 0.5 mL of B-PER, protein extraction reagents (Thermo Fisher Scientific, USA),
20 and 0.5 mL of 0.1 M acetate buffer was added to the solution to adjust the pH to 5.0. Then, the
21 solution was diluted with water to a total volume of 5 mL. Throughout the experiments, all sample
22 solutions were filtered using 0.45 µm membrane filters (mixed cellulose ester, DISMIC-25_{AS},
23 Advantec, Tokyo, Japan).
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48 2.3. Apparatus

49 An ICP-OES (Vista Pro, Seiko Instrument & Varian Instrument) was used for the
50 determination of the As species under conditions recommended by the manufacturer. A
51 laboratory-built pretreatment system, Auto-Pret, was employed for the successive measurements of
52 As(III), As(V), and their total concentrations. The system has been reported in previous papers
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1 [14-16]. Briefly, the system consisted of a syringe pump, an 8-port selection valve, and a 6-port
2 switching valve, all of which were controlled by a personal computer using in-house software written
3 by Visual Basic.
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7 A mini-column (2 mm i.d. × 40 mm, polypropylene) that was produced in-house was packed
8 with anion-exchange resin and was connected to the selection valve of the Auto-Pret. A schematic
9 illustration of the flow diagram is shown in Fig. 1. Initially, the mini-column was preconditioned
10 with 4 mL of 0.1 M sodium acetate solution. Then, 1 mL of a sample solution buffered at pH 5 was
11 introduced into the mini-column where As(III) was separated from As(V) when the As(V) was
12 retained onto anion-exchange resin (Fig. 1 (A)). The mini-column was then washed with 3 mL of
13 water for transport of the As(III) to ICP-OES. After the measurement of the As(III), 4 mL of 1 M
14 HNO₃ was introduced into the mini-column to elute and transport the As(V) to ICP-OES (Fig. 1 (B)).
15 Finally, the switching valve was switched so the sample would not pass through the mini-column and
16 another 1 mL of the sample was subjected to ICP-OES to determine the total concentration of As
17 (Fig. 1 (C)). The data obtained via ICP-OES, *i.e.*, the charts of time (s) vs. intensity (cps), were
18 processed *via* ICP-Expert, using ICP-OES Instrument Software V4.0 (Seiko Instruments, Chiba,
19 Japan) and an in-house LabVIEW program.
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41 **3. Results and discussion**

42 *3.1. Optimization of the pH for the sample and eluent*

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44 The separation of As(III) and As(V) was easily achieved by anion-exchange resin since the
45 acid dissociation constants, pK_{al} , of As(III) (arsenite) and As(V) (arsenate) were 9.22 and 2.22,
46 respectively [17]. Accordingly, the As(III) had no charge in the pH range of 0 to 7, whereas more
47 than 99% of the As(V) was anionic with a pH of more than 4.22. Therefore, if the pH of a sample
48 was adjusted to 5 with acetate buffer, only As(III) would pass through the anion-exchange column
49 due to the selective adsorption of As(V). After the As(III) had passed through the column, the
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1 As(V) could be eluted via the sequential flowing of an acidic solution. Conversely, to determine the
2 total As, we switched the switching valve so that the sample would not pass through the mini-column,
3 which resulted in a direct introduction of the sample into ICP-OES.
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7 For quantitative analysis of the As species, the pH of the sample solution and the
8 concentrations of the nitric acid used as an eluent for As(V) were varied for optimization. The pH
9 of the sample solution containing As(III) and As(V) was adjusted using 0.1 M acetate buffer
10 solutions with different pH values. At pH values of 4 to 6, the peak areas for As(III) were constant,
11 i.e., As(V) was completely retained on the mini-column even at pH 4. The nitric acid solutions with
12 concentrations that differed from 0.1 to 2 M were examined in order to elute the As(V) that had been
13 retained on the anion-exchange resin. The results showed that a concentration of more than 1 M
14 was sufficient to elute the As(V) from the mini-column. Consequently, the pH values of the sample
15 solutions were adjusted to 5.0 using the acetate buffer, then 1.0 M of nitric acid was employed to
16 elute the As(V) that had been retained on the anion-exchange resin. The measurements of the
17 As(III), As(V), and total As are summarized in Table 1. As shown in Table 1, the flow rate was
18 optimized at 30 $\mu\text{L/s}$ (1.8 mL/min), for which the total analysis time of one sample, including the
19 preconditioning step, was about 16 min.
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38 The present method has the following advantages; the instrument is much simpler than HPLC,
39 three species (As(III), As(V) and their total) are simultaneously determined, replacement of a
40 packing material is easy because of no need of high pressure for packing, and successive
41 measurements are automated by a program. So, the pretreatment system provides inexpensive,
42 reliable, and rapid analytical method for As speciation.
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53 3.2. Calibration curves

54 For the determination of each As species, calibration curves of As(III), As(V) and total As
55 were constructed under the optimized conditions described in section 3.1. The typical signals of the
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1 flow analysis are shown in Fig. 2. The sample solutions contained 0.2, 0.4 and 1.0 ppm of As(III)
2 and As(V) so that the totals were 0.4, 0.8 and 2.0 ppm, respectively. Fig. 2 shows the three
3 consecutive peaks of As(III) and As(V) with the totals in the same order. As noted in Fig. 2, As(V)
4 was detected as sharp peaks since it was concentrated on the mini-column. Conversely, the peak
5 width for As(III) and the totals were broader than that of As(V) due to the lack of preconcentration in
6 the mini-column. The standard solutions with different concentrations of As(III) and As(V) were
7 measured to construct the calibration curves, and the results are summarized in Table 2. At the
8 concentration range of 0–1.0 ppm, each calibration curve showed good linearity, while the slopes
9 were different. The change in the slopes of the calibration curves were attributed to the change in
10 the flow rate of each solution when introduced into ICP-OES, and to the preconcentration of As(V)
11 by the mini-column. In terms of the flow rate, we confirmed that the slope of the calibration curve
12 was reduced as the flow rate was increased. Although the flow rate was always controlled at 30 μL
13 s^{-1} , the mini-column increased the pressure, which resulted in a slightly decreased flow rate.
14 Therefore, during the measurement of total As, the flow rate was faster than it was for the As(III) and
15 As(V) measurements, resulting in a slope for total As that was smaller than that for As(III).
16 Conversely, the difference in the slopes between As(III) and As(V) was attributed to the
17 preconcentration of As(V) on the mini-column. The limits of detection (LOD) for the As species
18 (defined as the concentrations at a signal-to-noise ratio = 3 where the noise was defined as the
19 standard deviation of the background signals over a distance equal to 20 times the width at
20 half-height of the peak [18]) were estimated to be 158 ppb for As(III), 86 ppb for As(V) and 211 ppb
21 for total As. The LODs were slightly higher than those reported in the previous publications, e.g.,
22 10 ppb for As(III) and As(V) in HPLC-ICP-OES [3] and 0.1 ppb for As(III) and As(V) in ICP-OES
23 coupled with a preconcentration system [11]. However, the LODs obtained in the present method
24 were low enough to determine the concentrations of the As species in the culture media of *A.*
25 *brierleyi*.

3.3. Precipitation formation between As and Fe

Scordite, $\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$, is insoluble in water and its solubility product is $10^{-21.7}$ [19]. Therefore, when *A. brierleyi* produced Fe(III) and As(V), scordite or scordite-like ferric arsenate was simultaneously formed in the microorganisms, resulting in the immobilization of As species. To confirm the precipitation formation under the present culture conditions, 0–900 ppm of Fe(III) or Fe(II) solution was added to a mixture containing 200 ppm As(III) or 200 ppm As(V), respectively. Before the ICP-OES measurements, the sample solutions containing different concentrations of Fe(II) or Fe(III) were diluted 500-fold with the addition of 0.1 M acetate buffer. The concentration of each As species was plotted against the concentrations of Fe added to the samples, as shown in Fig. 3.

When Fe(III) was added to the mixture of As(III) and As(V), the concentration of As(V) was gradually decreased as the concentration of Fe(III) was increased due to the formation of precipitation between As(V) and Fe(III), while the concentration of As(III) was constant (Fig. 3(A)). Thus, the decrease in the total As concentration meant a selective precipitation of As(V) with Fe(III). Conversely, the concentrations of all As species remained constant when Fe(II) was added to the As mixture (Fig. 3(B)), i.e., no precipitation was formed in the presence of Fe(II). This means that the As and Fe must be As(V) and Fe(III) in order to form a precipitation of As and Fe as expected from the small solubility product of scordite.

3.4. Effect of Fe(II) on the oxidation of As(III) by *A. brierleyi*

Okibe and coworkers have demonstrated that the oxidation of As(III) to As(V) was enhanced in the presence of Fe(II), i.e., As(III) and Fe(II) were simultaneously oxidized by *A. brierleyi*, followed by the immobilization as precipitation of Fe(III) and As(V). *A. brierleyi* can oxidize Fe(II) to Fe(III) in its growth process, whereas the rate of the redox reaction between Fe(III) and As(III) was too small to detect As(V) in a medium containing Fe(III) [12] although Fe(III) can

1 oxidize As(III) to As(V), according to the standard redox potential for Fe(II)/Fe(III) and
2 As(III)/As(V) [17]. Therefore, As(III) should also be oxidized by *A. brierleyi*, as proposed by
3 Okibe and coworkers [12]. To confirm this simultaneous oxidation, two kinds of culture media
4 containing 500 ppm of As(III) were prepared, one with 900 ppm of Fe(II) and another without Fe(II).
5 After incubating *A. brierleyi* for 14 days, the concentrations of the As species in each medium were
6 determined by the proposed method. The results are shown in Fig. 4: (A) without Fe(II) and (B)
7 with Fe(II). In the absence of Fe(II), the peak area of As(III) was much larger than that of As(V),
8 i.e., the oxidation of As(III) would not take place or would proceed very slowly in the culture
9 medium. However, the peak area of As(V) increased as that of As(III) was decreased in the
10 presence of Fe(II). The total As concentrations were in good agreement with the sum of As(III) and
11 As(V) for both forms of culture media, that is, no loss of the As species was found in the
12 pretreatment system. Therefore, these results support those obtained by Okibe and coworkers who
13 found that the oxidation of As(III) by *A. brierleyi* was promoted by the presence of Fe(II) in the
14 culture medium.

3.5. Determination of the As species in the culture media and *A. brierleyi* cells

36 In the presence of *A. brierleyi*, As(III) was immobilized by the simultaneous oxidation of
37 As(III) and Fe(II) followed by the precipitation formation between As(V) and Fe(III). In addition,
38 Fe(II) was needed for the oxidation of As(III) to As(V) by *A. brierleyi*, although the reason remains
39 unclear. To obtain further information on the oxidation process, we attempted to determine the As
40 species in the culture media and the cell pellets at different incubation times. Fig. 5 shows the
41 dependence on incubation time of the As(III), As(V), and total As concentrations in the media and
42 cells. In the culture media, As(III) decreased rapidly and became constant after 6 days while As(V)
43 was almost constant. Since only As(III) was dissolved in the culture medium before incubation, the
44 decreased As(III) meant that the *A. brierleyi* had oxidized As(III) to As(V). The constant
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1 concentration of As(V) may be due to the exocytotic release of As(V) from the precipitation formed
2 in the cells. A similar decrement of As(III) in the culture media was observed in a recent study by
3 Okibe et al., although the residual amounts of As(III) were different from their results where As(III)
4 was completely oxidized after a 6-day incubation [20]. A possible reason may be a different sample
5 storage method, i.e., our samples were frozen for delivery in the present study while their samples
6 were stocked in a refrigerator and were determined within one week.
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14 It should be noted that the present study is the first demonstration of As speciation in *A.*
15 *brierleyi* cells. The results of the cell pellets indicated that the As species in the cells was only
16 As(V); i.e., As(III) was completely oxidized to As(V) in the cells after a 6-day incubation. The
17 total As concentration was in good agreement with the sum of As(III) and As(V) so that each species
18 was successfully determined by the proposed method. The results in Fig. 5(B) confirmed that As
19 was completely immobilized in the cells after 6 days [19], because no As(III) and only trace amounts
20 of As(V) were found in the cell pellets.
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31 The concentration of As(V) in the culture medium was almost constant at 100 ppm after a 6-day
32 incubation. The concentration was much lower than the solubility of scordite under culture
33 conditions of pH 1.0 since the solubility of scordite has been estimated at more than 10 mM, which
34 corresponds to 749 ppm [18]. Therefore, the precipitation of the ferric arsenate produced in *A.*
35 *brierleyi* would be immobilized in the cell body and/or extracellular polymeric substances region
36 without being exposed to the culture medium, as proposed by Okibe and coworkers [12]. The
37 results for the cell pellets also supported the proposed mechanism since no As (or a trace amount)
38 was found in the cell pellets after a 6-day incubation; i.e., As(III) was completely oxidized and was
39 immobilized in the cell body and/or extracellular polymeric substances region. Consequently, these
40 results suggest that the proposed method is applicable to the speciation of the As species in *A.*
41 *brierleyi* and its culture media.
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4. Conclusion

Here we developed a facile and reliable method for the determination of As(III), As(V), and total As via ICP-OES coupled with a flow-injection pretreatment system using a mini-column packed with an anion-exchange resin. The method was successfully applicable to the speciation of As in *A. brierleyi* cells and its culture medium. The results obtained in this study support the simultaneous oxidation of As(III) and Fe(II) as proposed in a previous study. The results for the determination of As species in cell pellets indicated that As(III) was completely oxidized to As(V) in the cultured *A. brierleyi* after a 6-day incubation. This fact is consistent with a previous report, which mentioned that As was immobilized as As(V) in *A. brierleyi*. Therefore, ICP-OES coupled with a flow-injection pretreatment system is a useful technique in the study of the oxidation mechanism of *A. brierleyi*, which is promising for use in the treatment of industrial and natural water containing inorganic As species.

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Table 1. Operation program for As speciation

Steps		Pump Valve	Selection Valve	Switching Valve*	Volume / μL	Flow rate / $\mu\text{L s}^{-1}$
Column washing	Aspirate	IN		1	1000	100
(Water)	Dispense	OUT	5		1000	30
Column conditioning	Aspirate		2		4000	100
(Sodium acetate)	Dispense		5		4000	30
Column washing	Aspirate	IN	2		4000	100
(Water)	Dispense	OUT	5		1000	30
Sample loading	Aspirate		3		1000	100
(Sample)	Dispense		5		4000	30
Eluent	Aspirate		4		4000	100
(Nitric acid)	Dispense		5		4000	30
Washing	Aspirate	IN		2	4000	100
(Water)	Dispense	OUT	5		1000	30
Total As	Aspirate		3		1000	100
(Sample)	Dispense		5		4000	30

*Switching Valve Position: 1; passing through the mini-column, 2; flowing to the ICP-OES directly

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Table 2. Calibration curves for As species.

Species	Concentration range/ ppm	Regression curve*	Correlation coefficient (R ²)
As(III)	0-1.0 (0.2 intervals)	y=95.8x	0.9937
As(V)	0-1.0 (0.2 intervals)	y=129x	0.9843
Total	0-2.0 (0.4 intervals)	Y=80.3x	0.9986

*The regression curves were obtained by fixing origin since no blank signal was observed for the sample without As.

Figure Captions

1
2 Figure 1. Flow diagram for the determination of As(III), As(V) and their total. (A) Introduction of
3 As(III) to ICP-OES, (B) introduction of As(V) to ICP-OES, and (C) introduction of total As to
4 ICP-OES.
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8 Figure 2. Typical profiles of flow analysis. a=0.2 ppm As(III), b=0.2 ppm As(V), c=0.4 ppm As,
9 d=0.4 ppm As(III), e=0.4 ppm As(V), f=0.8 ppm As, g=0.6 ppm As(III), h=0.6 ppm As(V), i=1.2
10 ppm As. The details of the experimental conditions are given in the text.
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14 Figure 3. Effect of iron on the precipitation of As species. The samples contained 200 ppm
15 As(III) and 200 ppm As(V). (A) Addition of Fe(II), (B) addition of Fe(III). Other conditions are
16 given in the text.
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21 Figure 4. Effect of Fe(II) on the oxidation of As(III) by *A. brierleyi*. The culture medium samples
22 contained 500 ppm As(III). The cells were incubated in the culture media (A) without Fe(II) and
23 (B) with Fe(II). Other conditions are given in the text.
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27 Figure 5. Speciation of As in *A. brierleyi* and its culture medium at different incubation times. *A.*
28 *brierleyi* was cultured in the media containing 500 ppm As(III) and 900 ppm Fe(II). The amounts
29 of As in the cells were divided by the number of the cells in order to correct the effect of the
30 differences in the cell population. Other conditions are given in the text.
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Figure 1

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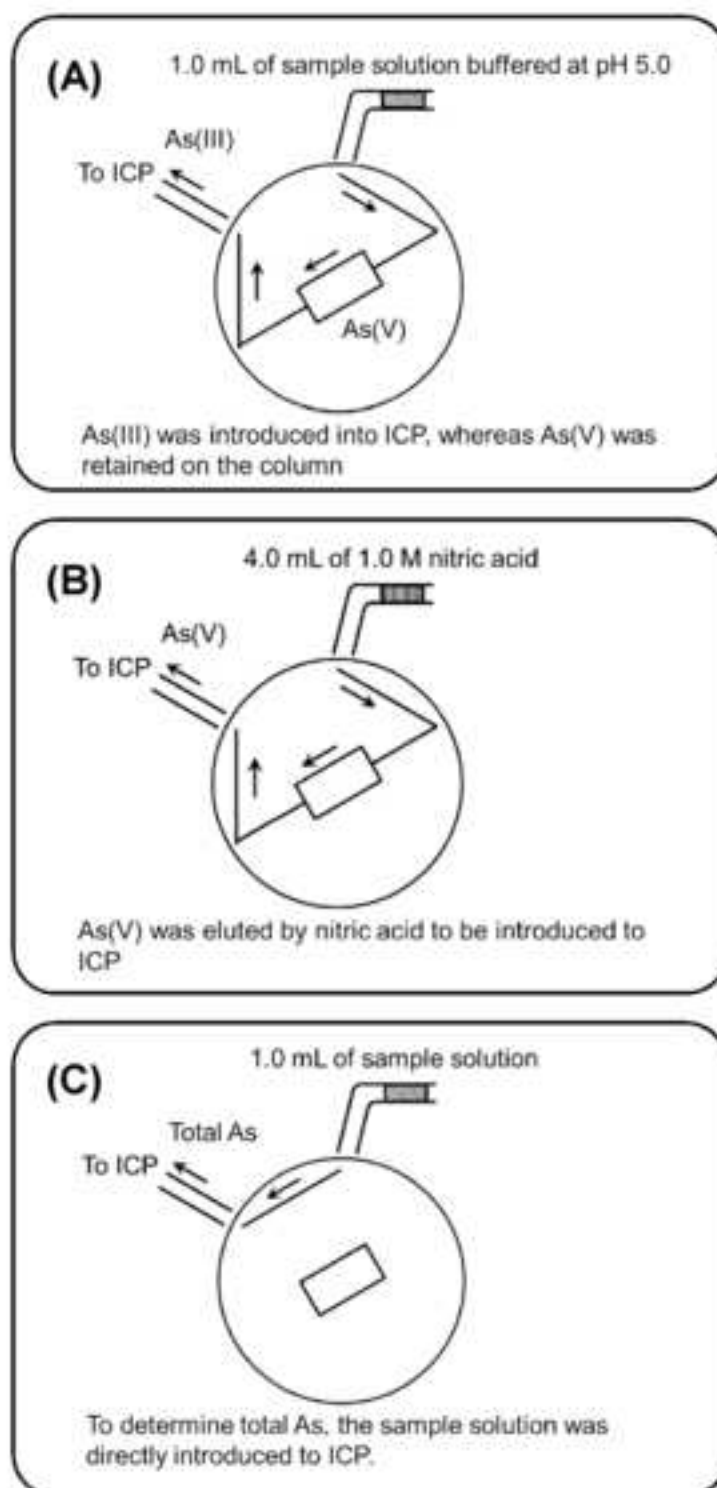


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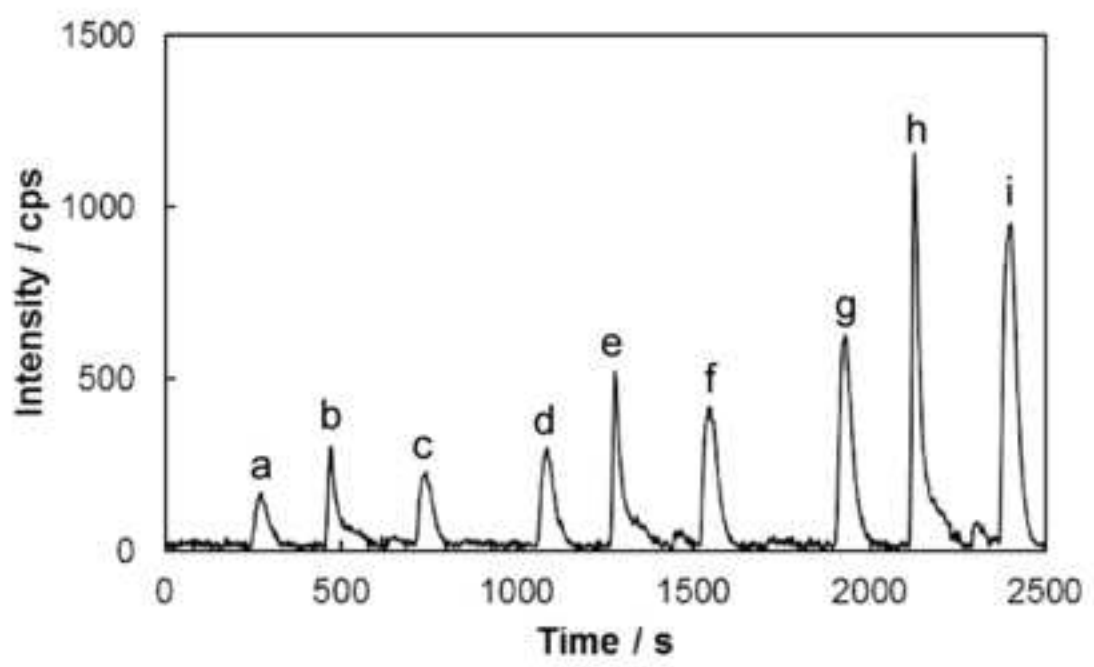


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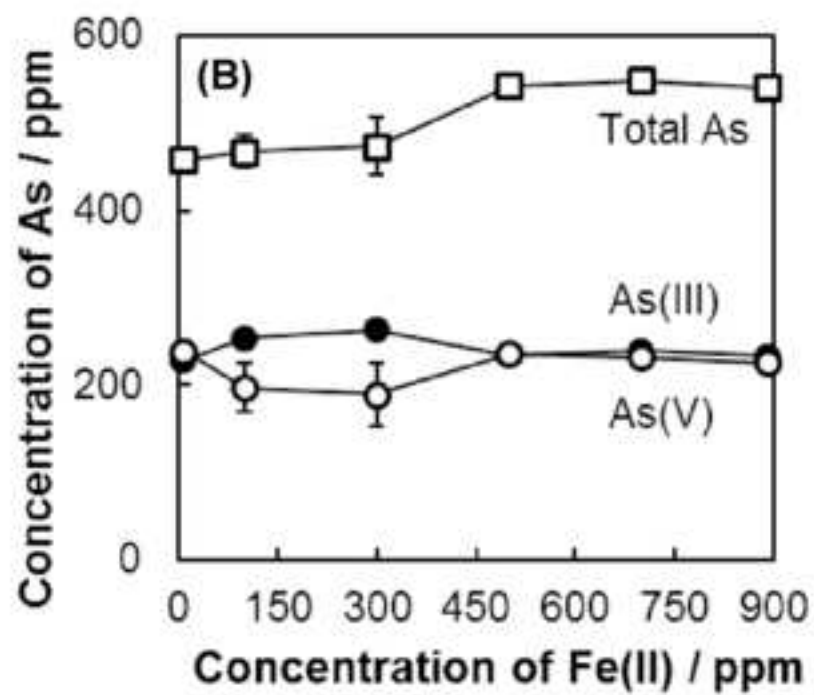
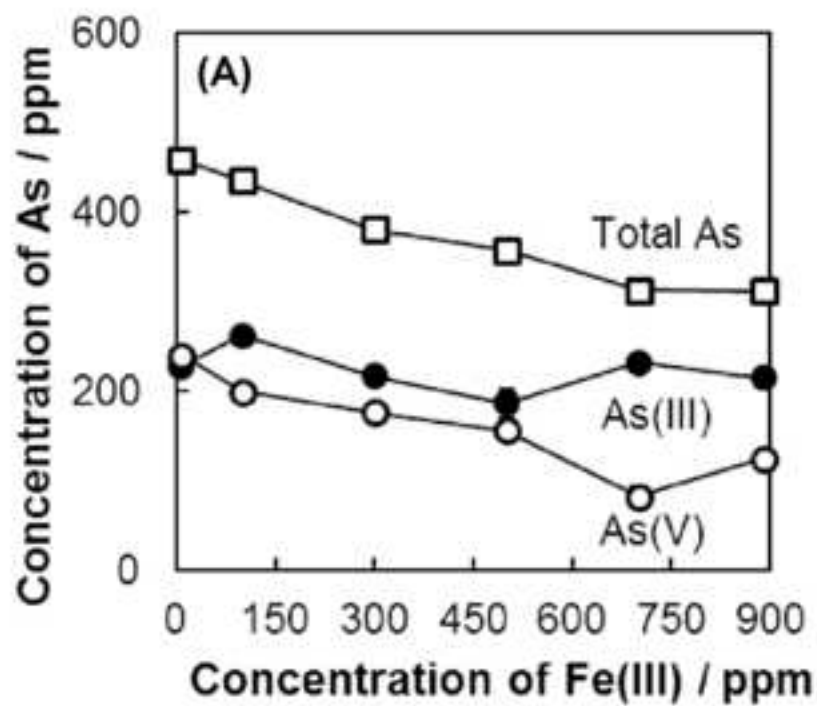


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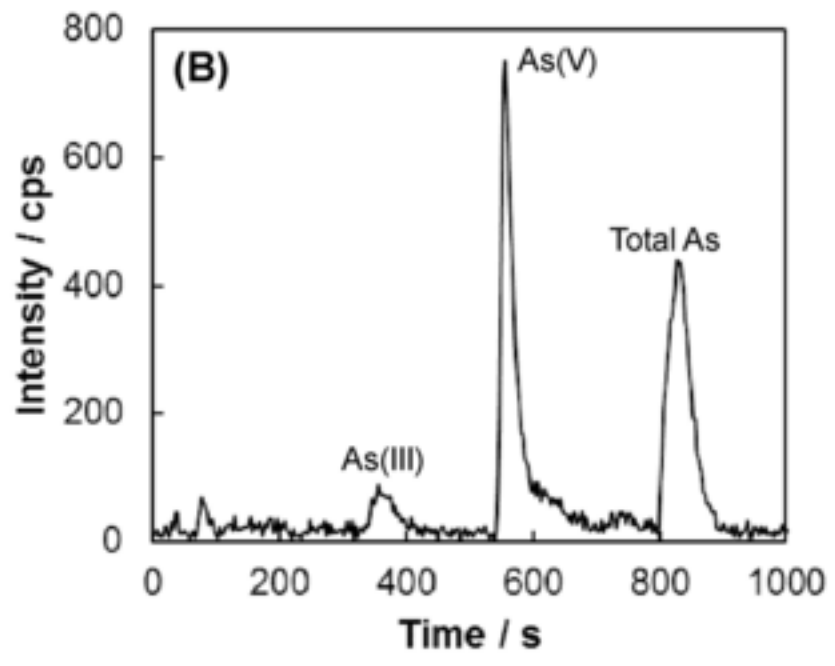
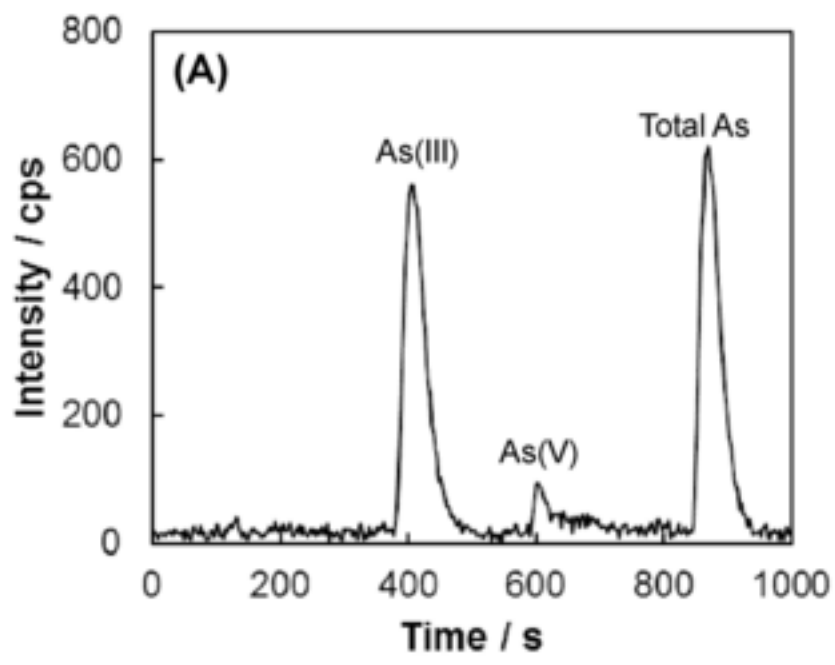


Figure 5
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