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# Antimony oxidation and adsorption by in-situ formed biogenic Mn oxide and Fe–Mn oxides

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## ABSTRACT

Antimony (Sb), which can be toxic at relatively low concentrations, may co-exist with Mn(II) and/or Fe(II) in some groundwater and surface water bodies. Here we investigated the potential oxidation and adsorption pathways of Sb (III and V) species in the presence of Mn(II) and Mn-oxidizing bacteria, with or without Fe(II). Batch experiments were conducted to determine the oxidation and adsorption characteristics of Sb species in the presence of biogenic Mn oxides (BMOs), which were formed *in-situ* via the oxidation of Mn(II) by a Mn-oxidizing bacterium (*Pseudomonas* sp. QJX-1). Results indicated that Sb(III) ions could be oxidized to Sb(V) ions by BMO, but only Sb(V) originating from Sb(III) oxidation was adsorbed effectively by BMO. Introduced Fe(II) was chemically oxidized to FeOOH, the precipitates of which mixed with BMO to form a new compound, biogenic Fe–Mn oxides (BFMO). The BMO part of the BFMO mainly oxidized and the FeOOH of the BFMO mainly adsorbed the Sb species. In aquatic solutions containing both As(III) and Sb(III), the BFMO that formed *in-situ* preferentially oxidized Sb over As but adsorbed As more efficiently. Chemical analysis and reverse transcription real-time polymerase chain reaction revealed that the presence of Fe(II), As(III) and Sb(III) accelerated the oxidation of Mn(II) but inhibited the activity of Mn-oxidizing bacteria. These results provide significant insights into the biogeochemical pathways of Sb, Mn(II) in aquatic ecosystems, with or without Fe(II).

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## Introduction

Antimony (Sb) is widely found in water and soil, with stibnite ( $\text{Sb}_2\text{S}_3$ ) being the most common mineral form (Lehr et al., 2007). Antimony is the ninth-most mined metal worldwide and is utilized in a wide array of industries ranging from chemical to pharmaceutical engineering (Abin and Hollibaugh, 2014). As a result of these multiple applications, Sb pollution from both natural and anthropogenic sources is

now a serious threat to drinking water quality and human health. The chemical and toxicological properties of Sb are similar to those of arsenic (As), which is not surprising given their proximity on the periodic table (Bentley and Chasteen, 2002). In both cases the trivalent form is responsible for the most prominent toxicological effects (Gebel, 1997). Despite the similarities, however, the environmental chemistries of the two elements are different. Little is known about the environmental transport and fate of Sb, whereas those of

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As have been extensively studied (Abin and Hollibaugh, 2014). In many locations worldwide, including China, high concentrations of As and Sb are often found together in some mining areas, leading to surface water or groundwater containing high levels of both contaminants (Asta et al., 2012; Luo et al., 2014).

Both Sb(III) and Sb(V), the two predominant ionic forms of Sb found in water, are identified as being acutely toxic, as well as carcinogenic. A drinking water standard of 5 µg total Sb per L has been suggested by the World Health Organization (2011). Achieving that concentration through treatment options can be both technically challenging and expensive. Technologies available for treating water with unacceptably high concentrations of metal ions include coagulation and flocculation, oxidation, membrane processes, electrochemical processes, and bioremediation (Ungureanu et al., 2015). The last named technique is often cited as the preferred method for treating Sb-polluted water owing to cost-effective outcomes. However, few data are available on the efficacy of biological treatment of Sb, and relatively little is known about the biologically mediated redox reactions and adsorption.

Manganese (Mn) oxides are ubiquitous in both aquatic and terrestrial soil environments where they play an important role in the biogeochemical cycling of organic and inorganic compounds (Butterfield et al., 2013). Mn(IV) oxide minerals have highly charged surfaces and are biogeochemically active, having the ability to lyse humic substances (Guo et al., 2009) and transform heavy metals as well as organics (Carmichael et al., 2013; Post, 1999). An increasing body of evidence has shown that microorganisms, especially bacteria, play a dominant role in the oxidation of dissolved Mn(II) in the natural environment (Geszvain et al., 2011). The rate of biological oxidation of Mn(II) has been demonstrated to be several orders of magnitude greater than natural chemical oxidation (Zouboulis and Katsoyiannis, 2004). In addition to a strong oxidizing ability, previous studies have also shown that the adsorption capacities of biogenic Mn oxides are greater than those of chemical Mn oxides (Katsoyiannis et al., 2004; Nelson et al., 1999; Tani et al., 2004; Tebo and Murray, 2007).

Fe(II) and Mn(II) ions are often found together in many groundwater and some surface water systems (Huang et al., 2012; Postawa and Hayes, 2013). In an oxygenated environment, Fe(II) may be oxidized to Fe(III) oxide, which mixes with biogenic Mn oxide (BMO) to form biogenic Fe–Mn oxide (BFMO). Previously we identified the structure of BFMO formed *in situ* (i.e., a mixture of MnO<sub>2</sub> and FeOOH), and found the BFMO had great oxidation and adsorption ability on As(III) and As(V) removal (Bai et al., 2016).

The goals of this study were to investigate the removal of Sb(III and V) as mediated by BMO and BFMO formed *in-situ*, as well as potential Sb oxidation and adsorption pathways. In addition, we assessed the competitive removal of As(III) and Sb(III) by BFMO in order to elucidate how the presence of other metalloid ions might influence Sb removal. The data generated in our study provide important information on biogeochemical cycling in aquatic ecosystems, as well as fundamental knowledge that may be applied to remediation of environments in which Sb is a significant pollutant.

## 1. Materials and methods

### 1.1. Mn(II)-oxidizing bacterium and culture medium

A Mn(II)-oxidizing bacterium, *Pseudomonas* sp. QJX-1 (GeneBank accession number, KM242057), was used in this study. The strain is able to oxidize Mn(II) at an optimum temperature of 30°C, and pH 7.5. The strain has been deposited with the China General Microorganism Culture Center (CGMCC; accession number, 6630).

A modified peptone–yeast extract–glucose (PYG) medium (1985) was used in the experiments. Each liter of PYG contained 0.25 g peptone, 0.25 g yeast extract, 0.25 g glucose, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, and 0.06 g CaCl<sub>2</sub>·2H<sub>2</sub>O. Fifteen micromole N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) was used as the buffer in the PYG medium. The pH was adjusted to 7.5 with 1 mol/L NaOH before autoclaving.

### 1.2. Oxidation and adsorption of Sb species by BMO and BFMO

A refrigerated culture of *Pseudomonas* sp. QJX-1 was activated in 100 mL PYG broth, shaken at 30°C and 170 r/min in a rotary shaker until the bacterial growth reached the logarithmic phase. Samples from the broth culture were washed three times by centrifugation with sterile deionized water and then used immediately in the following experiments.

A series of batch experiments were conducted in 500 mL-Erlenmeyer flasks to compare Sb removal by BMO and BFMO. For the BMO experiment, each flask contained 200 mL of PYG medium containing approximately 100 µmol/L Mn(II) as MnCl<sub>2</sub>, and 10 µmol/L Sb(III and V) added as potassium antimony tartrate (C<sub>4</sub>H<sub>4</sub>KO<sub>7</sub>Sb·1/2 H<sub>2</sub>O) and antimonate dipotassium trihydrate (KSbO<sub>3</sub>·3H<sub>2</sub>O). A flask without the bacterial inoculum was used as a negative control under the same conditions. All flasks were sealed with seal film, shaken at 30°C and 170 r/min, and sampled periodically. The OD<sub>600</sub> values reflecting the bacterial growth were measured against time. To analyze the concentrations of Mn(II), Sb(III and V), a portion of the sample was filtered through a 0.22-µm membrane. The pH values were measured at the beginning and at the end of the experiment. For the BFMO experiments, we first explored the optimal initial Mn(II)/Fe(II) ratio for Sb(III and V) removal, different initial concentrations of Fe(II) in the form of FeSO<sub>4</sub>·7H<sub>2</sub>O were added to the PYG media with Mn(II) and Sb(III or V). Then we added the bacteria QJX-1 to the media. After 72 hr, the BFMO had already formed and the Sb(III and V) oxidation and adsorption completed. Samples were taken for chemical analysis (Fe, Mn and Sb species concentrations). From the results of these determinations, we deduced the optimum initial Mn(II)/Fe(II) ratio for Sb removal, which we used to investigate the oxidation and adsorption processes. The experimental procedure for exploring the oxidation and adsorption processes was the same as for the BMO experiment, except that Fe(II) was in the reaction mixture. In addition, Sb oxidation and adsorption by Fe oxide was also tested, i.e., only adding Fe(II) in the media containing Sb(III) or Sb(V). After shaking and fully contacting with oxygen, the Fe(II) would be quickly transformed into Fe oxide and the Sb oxidation and adsorption occurred. The results

were compared with those from the BFMO experiment. Because the Fe(II) cannot be transformed or adsorbed (<3%) by QJX-1 (data not shown), we did not perform Sb oxidation and adsorption assay in the media containing Sb, Fe(II) and QJX1 for comparison.

### 1.3. Oxidation and adsorption of As and Sb complexes by BFMO

This experiment focused on the competitive oxidation and adsorption of As and Sb by the BFMO. Approximately 10  $\mu\text{mol/L}$  As(III) (prepared by sodium arsenite ( $\text{NaAsO}_2$ )) and Sb(III) were added to the PYG medium containing 100  $\mu\text{mol/L}$  Mn(II). QJX-1 was added next and the suspension sampled at regular intervals. The samples were filtered and then analyzed for Mn(II), As(III), Sb(III), and total As and Sb concentrations. Because growth measurements at  $\text{OD}_{600}$  were not possible due to the interference by the coprecipitation with BFMO, we used RT-realtime PCR (reverse transcription real-time polymerase chain reaction) to measure 16S rRNA (ribosomal RNA) expression over time.

### 1.4. Chemical analyses

The  $\text{OD}_{600}$  of cell suspensions was measured with an ultraviolet spectrophotometer (Hitachi U-3010, Japan). For determinations of Mn(II), Fe(II), and total Sb and As in solution, samples of suspension were first filtered through a 0.22  $\mu\text{m}$ -membrane and the filtrate adjusted to  $\text{pH} < 2$  with 1 mol/L HCl. They were then assayed by inductively coupled plasma/optical emission spectroscopy (ICP-OES, Agilent 700 series, USA). For determinations of Sb(III) and As(III), samples of suspension were filtered through a 0.22  $\mu\text{m}$  membrane and the filtrate analyzed by atomic fluorescence spectrometry (AF-610B, Beijing Rayleigh Analytical Instrument Co., China). Sb(V) and As(V) concentrations in solution were calculated by subtracting the Sb(III) and As(III) values from the total Sb and As concentration values.

We also collected the BMO or BFMO precipitations with or without adding Sb in the media (at 72 hr). The precipitations were washed for three times using aseptic ultra-pure water in order to remove Sb and other ions in the solution. The structural characteristics (morphology and element distribution) of BMO and BFMO were captured by a field emission scanning electron microscopy (SEM; Hitachi SU-8020, Japan). The X-ray photoelectron spectroscopy (XPS) technique was used for the structural analysis. XPS analysis was performed with a Kratos AXIS Ultra X-ray photoelectron spectrometer equipped with a monochromatized Al K $\alpha$  X-ray source (Bai et al., 2013).

### 1.5. Molecular analysis

In the experiment of competitive oxidation and adsorption of As(III) and Sb(III) by BFMO, total RNA was extracted from bacterial suspension samples using a TRNzol Reagent Kit (Tiangen Biotech Co. Ltd., Beijing, China), according to the manufacturer's instructions. The total RNA was immediately reverse transcribed with a Tiangen QuantiScript reverse transcription (RT) Kit (Tiangen Biotech Co. Ltd., China). The

synthesized cDNA samples were stored at  $-20^\circ\text{C}$  until use. The bacterial 16S rRNA gene of *Pseudomonas* sp. QJX-1 was quantified by an ABI 7300 real-time PCR system based on the SYBR Green I method. The primer pairs were designed according to the 16S rRNA sequence of QJX-1. Their respective nucleotide sequences were as follows (the PCR products consisted of ~200 bp): 16S rRNA-F, 5'-TGTAGCGGTGAAATGCGTAG-3' and 16S rRNA-R, 5'-CCCAGGCGGTCAACTTAAT-3'. The thermal program for the real-time PCR was 30 sec at  $95^\circ\text{C}$ ; 40 cycles of 15 sec at  $95^\circ\text{C}$ , 30 sec at  $55^\circ\text{C}$ , and 1 min at  $60^\circ\text{C}$ ; followed by 15 sec at  $95^\circ\text{C}$  and 1 min at  $60^\circ\text{C}$ . The plasmid standard for the 16S rRNA gene was generated using our previous method (Bai et al., 2012). The specificity of the PCR for target gene was checked using melting curve analysis and gel electrophoresis.

## 2. Results

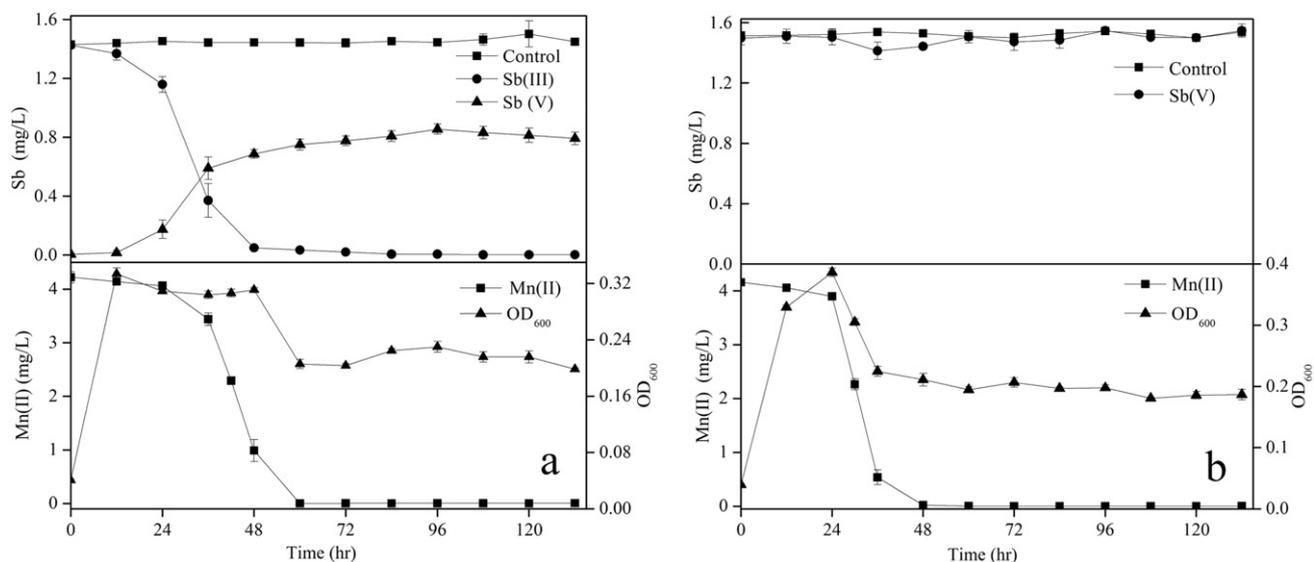
### 2.1. Sb oxidation and adsorption by BMO

In a media containing Mn(II), Sb species and QJX1, BMO was quickly formed by stationary phase cells of strain QJX-1 in the media containing Mn(II) and Sb species (Fig. 1). Along with the formation of BMO, (96.66  $\pm$  0.45)% of Sb(III) was oxidized to Sb(V) by BMO within 48 hr (Fig. 1a). The resulting Sb(V) was immediately adsorbed by the BMO. Although desorption occurred concurrently with adsorption, (44.38  $\pm$  2.99)% of Sb was still adsorbed on the BMO surface after 132 hr, corresponding to a removal capacity of (0.15  $\pm$  0.01) mg total Sb/mg total Mn. In contrast to Sb(III), only (5.65  $\pm$  1.40)% of Sb(V) was directly adsorbed by the BMO at 36 hr (Fig. 1b), and most of the adsorbed Sb(V) was released again into solution during the subsequent desorption process.

The structure of the surface of BMO and of BMO with adsorbed Sb species was examined by SEM-EDX and XPS. Because of the weak adsorption capacity of BMO for Sb(V), a detectable signal for Sb(V) at the surface of BMO was not obtained. Therefore, we only compared the surface structures of BMO and BMO-Sb (from Sb(III) medium), as shown in Fig. S1. EDX revealed that the elements Mn, C and O were present at the surface of BMO. After adsorption had occurred, Sb was also detected in addition to the previously mentioned Mn, C, and O at the surface of the Mn oxide. XPS analysis revealed a binding energy of 642.1 eV, which indicates the Mn oxide peak was higher than that of MnO (640.6 eV, +2 valence state) (Crowther et al., 1983) and MnOOH (641.5 eV, +3) (Nesbitt and Banerjee, 1998), suggesting a valence of Mn in BMO that falls between III and IV. The peak at 532.6 eV was indicative of the existence of Sb(V) at the surface of the Mn(IV) oxide (Wang et al., 1991).

### 2.2. Sb oxidation and adsorption by BFMO

We investigated the formation of BFMO and its role in Sb oxidation and adsorption. Multiple treatments were performed, each with a different concentration of Fe(II). The Fe(II) was introduced into PGY medium containing specified Mn(II) and Sb species. Mn-oxidizing bacteria were subsequently added. Aeration of the inoculated medium cause rapid oxidation of Fe(II) to Fe(III) oxide (FeOOH). The resulting

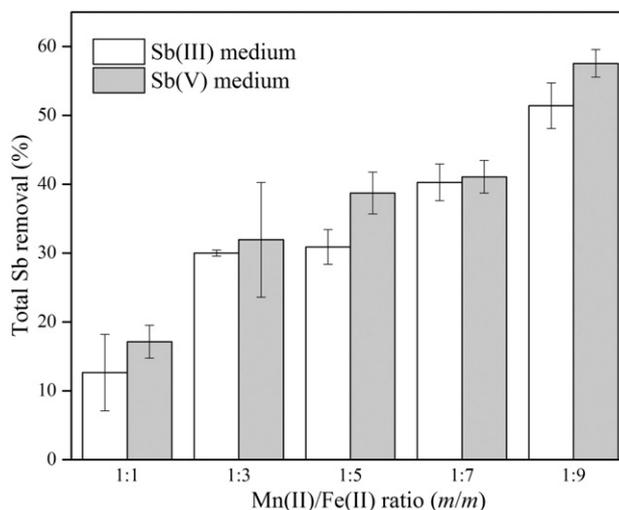


**Fig. 1 – Oxidation and adsorption of Sb by manganese oxide formed *in-situ*. The experiment was conducted in the PYG media containing Sb(III or V) and Mn(II), and then the cultured QJX-1 was added. (a) Sb(III) + Mn(II) + QJX1 in the media, (b) Sb(V) + Mn(II) + QJX1 in the media, control: QJX-1 + Sb(III) or Sb(V), but without Mn(II) in the media. Each data point represents a mean ± standard deviation (n = 3). PYG: peptone–yeast extract–glucose.**

mixture was combined with BMO, forming BFMO. More than 94% of Fe(II) and Mn(II) were oxidized and formed the BFMO (Fig. S2), and more than 99% of Sb(III) was oxidized to Sb(V) at all Mn(II)/Fe(II) ratios at which the BFMO was formed (Fig. S3). Total Sb removal by BFMO under varying initial Fe(II) concentrations is illustrated in Fig. 2. The removal capacities of the BFMOs are shown in Table 1. An increase in total Sb removal correlated with an increase in initial Mn(II)/Fe(II) ratios. Removal was found to be greater in test medium containing Sb(V) than in medium containing Sb(III). Solution pH during the experiment remained within a relatively narrow neutral range of 7.0–7.5, indicating that Sb removal was mediated by BFMO adsorption, not by chemical precipitation. Compared to BMO, the Sb-removal capacity of BFMO was significantly improved in the Sb(V) reaction system. The optimum removal capacity for Sb species occurred when the initial Mn(II):Fe(II) ratio was 1:3 (Table 1). SEM–EDX analysis revealed that Sb(V) was present at the BFMO surfaces, both in Sb(III) and Sb(V) reaction systems (Fig. S4).

Considering the optimum Mn/Fe ratios for Sb removal (1:3), we further investigated the oxidation and adsorption process of Sb species by *in-situ* formed BFMO and Fe oxide (FeOOH). Chemical oxidation of Fe(II) and biological oxidation of Mn(II) occurred simultaneously (at around 30 hr; Fig. 3a and b). The rate of chemical Fe(II) oxidation in the medium containing Sb(III) was noticeably slower than in the medium containing Sb(V). As the data for Fe(II) oxidation in the presence of BFMO (Fig. 3a and b) and in the presence of Fe oxide alone (Fig. 3c and d) show, BMO was apparently able to oxidize residual Fe(II) to Fe oxide, because the formation of Fe oxide was enhanced in the presence of BFMO (5%–10%). Along with the formation of Fe oxide and BMO, BFMO was generated and Sb(III) oxidation and Sb(III) and V adsorption was initiated. While BMO oxidized Sb(III) more efficiently than did Fe oxide

(Fig. 3a and c), the adsorption of Sb(III and V) by Fe oxide was significantly greater than by either BFMO or Mn oxide alone. Adsorption of Sb species by FeOOH was very rapid. FeOOH adsorbed 71.95% of Sb(III) and 97.51% of Sb(V) on its surface within 1 hr (Fig. 3c and d).



**Fig. 2 – Total Sb removal by *in-situ* formed BFMO in the medium containing Sb(III) or Sb(V). The initial concentration of the Sb(III) or Sb(V) species was 10 μmol/L, and initial Mn(II) concentration was 5 mg/L. Different initial Fe(II) (5, 15, 25, 35, 45 mg/L) were added into the medium. Samples were obtained at 72 hr. In the figure, Sb(III) medium denotes Sb(III) + Mn(II) + Fe(II) + QJX1 in the medium, and Sb(V) medium denotes Sb(V) + Mn(II) + Fe(II) + QJX1 in the medium. Each data point represents a mean ± standard deviation (n = 3). BFMO: biogenic Fe–Mn oxides.**

**Table 1 – Capacity of BFMOs with different initial Mn(II)/Fe(II) ratios to remove Sb species .**

Mn/Fe ratio (m/m)	Sb(III)	Sb(V)
1:1	0.021 ± 0.009	0.034 ± 0.005
1:3	0.033 ± 0.001	0.042 ± 0.011
1:5	0.021 ± 0.002	0.031 ± 0.002
1:7	0.020 ± 0.001	0.023 ± 0.001
1:9	0.019 ± 0.001	0.025 ± 0.001

\* The unit is mg total Sb/mg total Fe + Mn. The removal capacities were calculated by (Initial Sb – Remaining Sb in the media at 72 hr)/Initial adding Fe + Mn amounts. Data points denote mean ± standard deviation (n = 3).

### 2.3. Oxidation and adsorption of As and Sb complexes by BFMO

In the natural environment, As(III) and Sb(III) are much more toxic than As(V) and Sb(V), respectively. This experiment was conducted to simulate the biogeochemical process in aquatic ecosystems when the water containing the four metal ions (Fe(II), Mn(II), As(III) and Sb(III)) and explore the competitive oxidation and adsorption of As(III) and Sb(III) by *in-situ* formed BFMO. From previous study, the QJX cannot utilize and adsorbed As (Bai et al., 2016). The results (Fig. 4a) showed that the Mn(II) oxidation rate in PYG medium containing Fe(II), Mn(II), As(III), and Sb(III) was significantly greater than that in the medium containing only Mn(II) (t test,  $p < 0.01$ ). When As(III) and Sb(III) were both present in the medium, As(III) and Sb(III) were oxidized to As(V) and Sb(V), respectively, which were then adsorbed on the BFMO surface (Fig. 4b). The Sb(III) oxidation (96.36%) was more efficient than As(III) oxidation (84.71%). But the As adsorption by the BFMO ((0.026 ± 0.001) mg total As/total mg Fe and Mn) was significant higher ( $p < 0.01$ , paired t-test) than the Sb adsorption ((0.013 ± 0.001) mg total Sb/mg total Fe and Mn).

To explore the activity of QJX-1 during the Mn oxidation, we investigated the 16S rRNA gene expression during the Mn oxidation reaction in the medium containing Mn(II), Fe(II), As(III), and Sb(III). Medium containing only Mn(II) was used as a control (Fig. 5). While the reaction was proceeding (0–46 hr), the 16S rRNA gene expression in the medium containing only Mn(II) was significantly greater ( $p < 0.01$ , Wilcoxon Signed Ranked Test) than that in the medium containing Mn(II), Fe(II), As(III) and Sb(III). In addition, the time point of maximum expression was earlier in the medium only containing Mn(II) (10 hr for the medium containing only Mn(II); 22 hr for the medium containing Mn(II), Fe(II), As(III), and Sb(III)). These results revealed that bacterial activity may be inhibited by the presence of Fe(II), As(III), and Sb(III).

## 3. Discussion

According to predictions based on thermodynamic equilibria, Sb(V) occurs primarily in well oxygenated aquatic environments, whereas Sb(III) occurs in oxygen-depleted environments (Filella and May, 2003). However, substantially elevated concentrations of Sb(III) in oxic waters and Sb(V) in anoxic waters have been frequently observed (Filella et al., 2002;

Quentel et al., 2006). Under the pH conditions (7.0–7.5) used in this study, Sb(III) should normally form a neutral complex, Sb(OH)<sub>3</sub>, whereas Sb(V) should exist as a negatively-charged complex, Sb(OH)<sub>6</sub><sup>−</sup> (Filella and May, 2005).

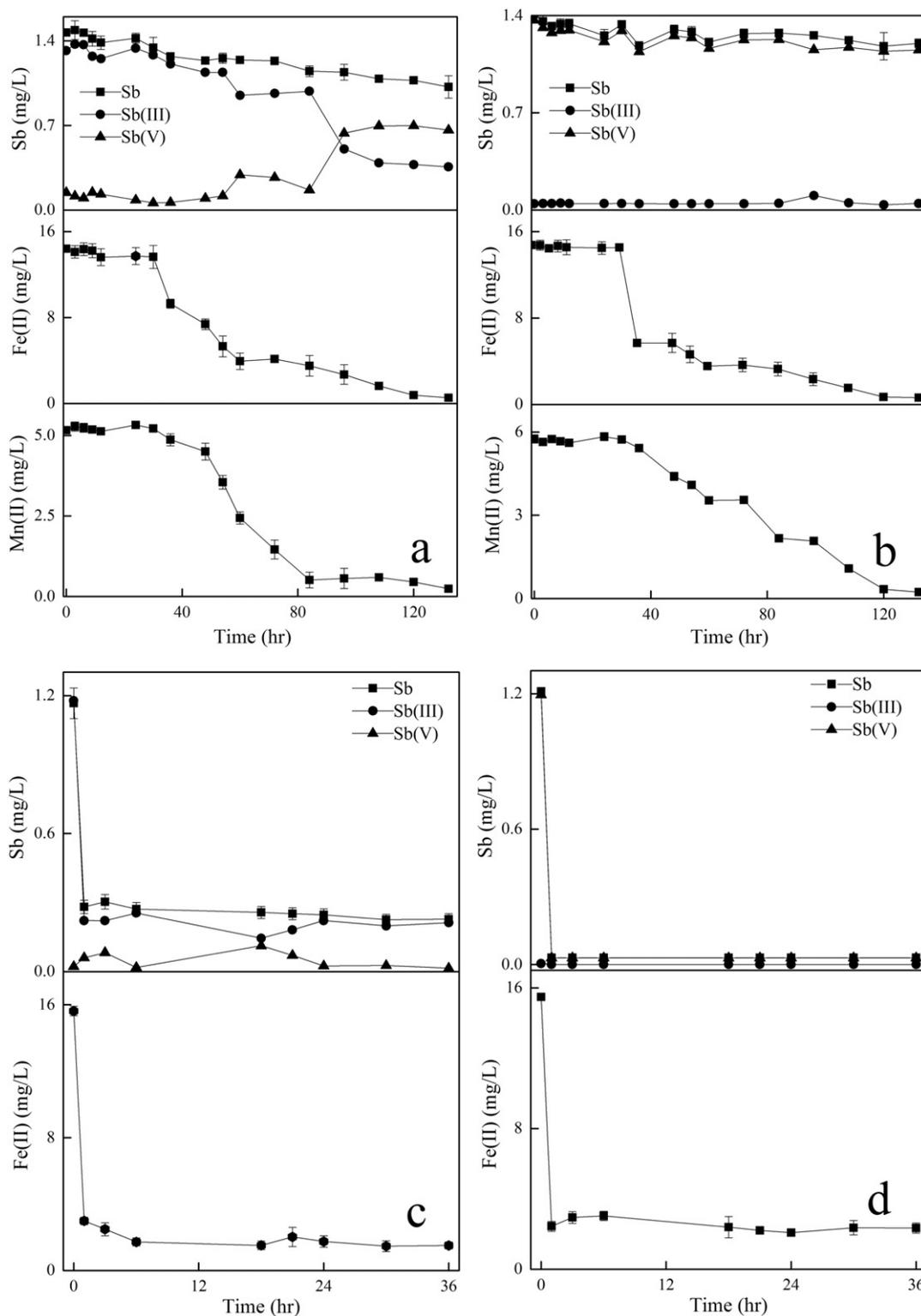
### 3.1. Mechanism of Sb removal by BMO and BFMO

Although previous studies have shown that various organic and inorganic chemicals will significantly adsorb to BMO due to its highly charged surfaces (Tebo et al., 2010), our study demonstrated that the adsorption capacity of BMO is strongly related to the valence and types of adsorbed materials. BMO can oxidize and adsorb Sb(III) efficiently but the adsorption of Sb(V) is comparatively lower. Previous research also demonstrated that Sb(III) sorbs more strongly than Sb(V) to surfaces such as those of Fe(III)- and Mn(IV)-(hydr)oxides (Thanabalasingam and Pickering, 1990). Tendencies may be explained by the negative charge of BMO, which would repulse negatively charged Sb(V) (i.e., Sb(OH)<sub>6</sub><sup>−</sup>). However, the negatively-charged BMO surface would have only weak electrostatic attraction for Sb(III) in the form of neutral Sb(OH)<sub>3</sub>. Therefore, it is likely that significant adsorption of Sb(III) to BMO is caused by the stable ligand formed by the Sb(OH)<sub>3</sub>/BMO complex. Although Sb(III) was oxidized to Sb(V) through the action of BMO, desorption was not significant, unlike previous findings involving Sb(III) adsorption by goethite (Leuz et al., 2006; Watkins et al., 2006) and bentonite (Xi et al., 2011). This suggests that the binding force between Mn oxide surface sites and Sb(III) was high enough to overcome the repulsive charge effect (Thanabalasingam and Pickering, 1990), and BMO's adsorption of Sb(III) was probably via inner-sphere electron transfer.

As has been reported in similar studies, the rapid manner in which Sb(III) and Sb(V) were adsorbed to the surface of *in-situ* formed Fe oxides was likely due to the shared characteristic of forming inner-sphere complexes at the surface of Fe oxide (Leuz et al., 2006; Xi et al., 2013). In BFMO, Mn oxide was the dominant driver in the oxidation of Sb(III) to Sb(V), whereas Fe oxide was mainly responsible for the adsorption of Sb. However, when Mn oxide was present, adsorption of Sb by Fe oxides (FeOOH) was diminished in both Sb(III) and Sb(V) systems. In the Sb(III) system, the observation that Sb adsorption by Fe–Mn oxides was lower than adsorption by BMO and Fe oxides was surprising. This occurrence was probably due to the Mn ions occupying FeOOH surface sites and forming the Fe–O–Mn complex, resulting in the significant decrease of Sb adsorption from BMO and Fe oxide.

### 3.2. Comparison of BMO with synthetic Mn oxide, and BFMO with synthetic Fe–Mn binary oxides

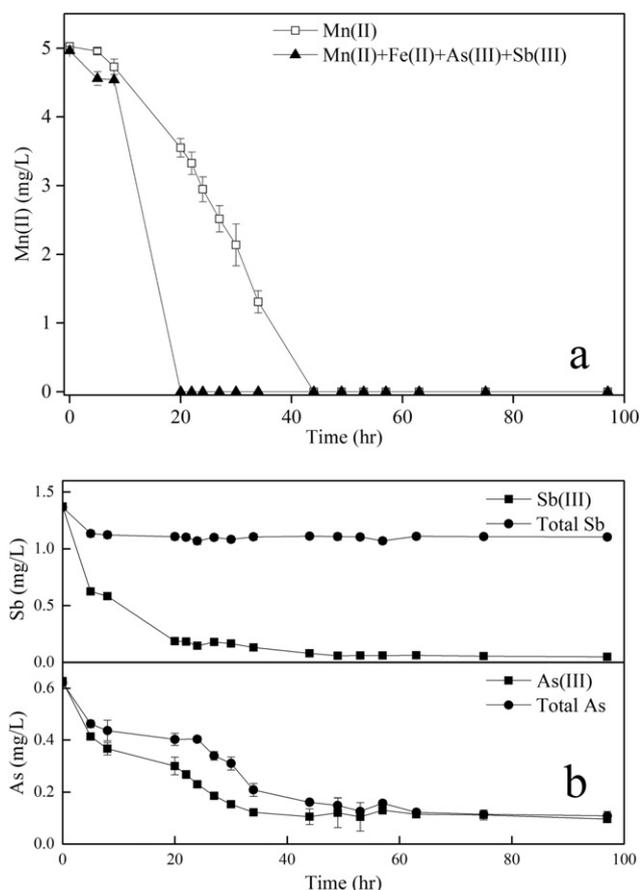
We previously proved that the BMO formed is structurally similar to birnessite and the FMBO is a mixture of MnO<sub>2</sub> and FeOOH (structurally similar to lepidocrocite) (Bai et al., 2016). Previous studies have reported that the synthetic Mn oxide (e.g., gamma-MnOOH) could oxidize Sb(III) within minutes, and that the adsorption of Sb(V) by manganite was an exothermic process (Wang et al., 2012). While BMO acts in much the same way, there are two key differences between chemical Mn oxide and BMO regarding the adsorption of Sb species, those being (1) chemical synthetic Mn oxide have a



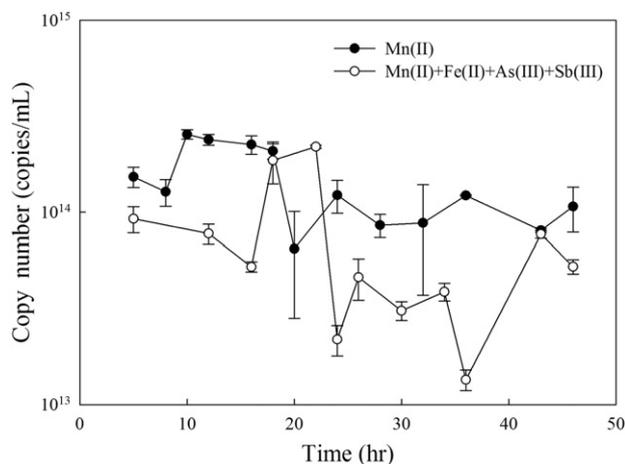
**Fig. 3 – Oxidation and adsorption of Sb by BFMO and Fe oxide. (a) Sb(III)–BFMO; (b) Sb(V)–BFMO; (c) Sb(III)–FeOOH; and (d) Sb(V)–FeOOH. Initial Mn(II)/Fe(II) in the PYG media is 1:3 (*m/m*). Each data point represents a mean ± standard deviation (*n* = 3).**

greater capacity to adsorb Sb(V); (2) upon oxidation by Mn oxide, Mn(II) from the reduction of Mn oxide would be released into solution. For the *in-situ* BMO system, bacteria would oxidize the Mn(II) ion to BMO again and little Mn(II) would be present in solution. With a synthetic Mn oxide system, however, Mn(II) would remain in solution.

Synthetic Fe–Mn binary oxides have demonstrated their ability to remove various organic or inorganic pollutants (Liu et al., 2012; Zhang et al., 2007, 2009). BFMO has similar oxidative (mainly from the Mn oxide) and adsorptive (mainly from Fe oxide) properties. While the Fe–Mn binary oxides release Fe(II) and Mn(II) during the oxidation of Sb(III), BFMO



**Fig. 4 – Oxidation and adsorption of As(III) and Sb(III) complexes by in-situ formed BFMO. (a) Mn oxidation in the media containing Mn(II), Fe(II), As(III), and Sb(III), and in the media containing only Mn(II) (control); (b) Sb and As oxidation and adsorption. Initial Mn(II)/Fe(II) in the PYG media was 1:3 (*m/m*). Each data point represents a mean  $\pm$  standard deviation ( $n = 3$ ).**



**Fig. 5 – The comparison of 16S rRNA gene expression between in the PYG medium containing Mn(II) + QJX1 and in the PYG media containing Mn(II) + Fe(II) + As(III) + Sb(III). The data were obtained from RT-realtime PCR assay. Each data point represents a mean  $\pm$  standard deviation ( $n = 3$ ).**

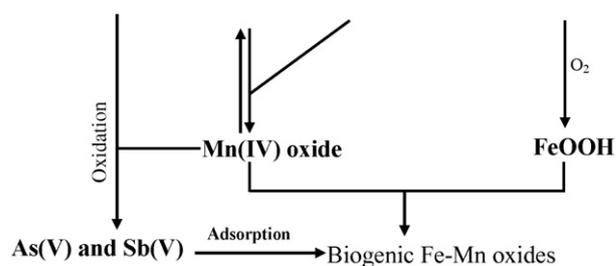
does not, which provides BFMO with a distinct advantage. Conversely, use of BFMO has a disadvantage in that Mn oxide and some bacteria occupying the adsorption sites located on the FeOOH surface, decrease adsorptive capacity when compared to the chemical Fe–Mn binary oxides. As a result, the chemical Fe–Mn binary complex demonstrates greater Sb removal capacity relative to BFMO (Xu et al., 2011).

### 3.3. Competitive oxidation and adsorption of As(III) and Sb(III) by BFMO

Mining of hydrothermal Sb ores is an important source of significant Sb and As contamination in the environment (Hiller et al., 2012). In the mine area, Sb(III), As(III), Fe(II) and Mn(II) may coexist in surface water or groundwater. In surface water ecosystems where water is generally well-oxygenated or when groundwater is pumped for drinking water, BFMO may form either directly in the natural water body or in the drinking water treatment system (e.g., sand filter). Based on the information generated in the current study, as well as on our previous results for As oxidation and adsorption by BFMO (Bai et al., 2016), we propose a potential pathway for As(III) and Sb(III) oxidation and adsorption when Mn(II) and Fe(II) coexist in water (Fig. 6). In summary, when a Mn-oxidizing microbe (*Pseudomonas* sp. QJX-1) is introduced into a solution, soluble Mn(II) is oxidized to insoluble Mn(IV) oxide; if Fe(II) is present, biogenic Fe–Mn oxides (BFMO) are formed in an oxygenated environment. BFMO that is formed in-situ could then oxidize and further adsorb the As and Sb species.

The experimental results (Figs. 4 and 5) revealed that the presence of Fe(II), As(III) and Sb(III) facilitated Mn(II) oxidation, but inhibited bacterial growth. In combination with the evidence that Mn oxidation activity occurred during the stagnant phase of bacterial growth (Fig. 1), the results indicate that QJX-1 plays an indirect role in Mn oxidation, specifically, bacteria first produced a substance (probably a superoxide) which catalyzed the formation of Mn oxide (Hansel et al., 2012; Learman et al., 2013). Furthermore, we found that an oligotrophic medium may accelerate the initiation of Mn oxidation that is induced by QJX1 (Fig. S5).

#### As(III) and Sb(III)+Mn(II)+Mn-oxidizing microbe+ Fe(II)



**Fig. 6 – Proposed As(III) and Sb(III) oxidation and adsorption pathways by in-situ formed BFMO. Mn(II) is first oxidized to Mn(IV) by Mn-oxidizing microbe, and Fe(II) is chemically oxidized to FeOOH. Mn(IV) oxide and FeOOH are mixed to form a new compound: biogenic Fe–Mn oxides (BFMO). For As(III) and Sb(III) removal, As(III) and Sb(III) are first oxidized to As(V) and Sb(V), mainly by biogenic Mn(IV) oxide. They are then adsorbed by BFMO.**

These results suggest the possibility of utilizing BMO or BFMO (if Fe(II) is present) as a water purification factor in oligotrophic environments such as groundwater.

#### 4. Conclusions

In summary, we reached three primary conclusions from our research. (1) In the Mn(II)-Sb(III)-manganese oxidizing microbe system, the resulting BMO is able to oxidize Sb(III) to Sb(V) and to further adsorb Sb(V) to its surface ( $0.15 \pm 0.01$  mg total Sb/mg total Mn). However, in the Mn(II)-Sb(V)-manganese oxidizing microbe system, the Sb(V) ion is not significantly adsorbed by BMO due to the electrostatic charge repulsion. (2) Upon the introduction of Fe(II), BFMO (originating from the mixing of BMO and FeOOH produced from chemical oxidation), will oxidize Sb(III) and improve Sb(V) adsorption. The optimum adsorption capacity for Sb(III and V) occurred when the initial Mn(II):Fe(II) ratio was 1:3. In a manner similar to the synthetic Fe–Mn binary oxides, the Mn oxide portion of BFMO is primarily responsible for the oxidation of Sb(III) and the Fe oxide portion is responsible for Sb adsorption. However, the BFMO action is different from synthetic Fe–Mn binary oxides in that the Mn(II) and Fe(II) ions would not be released to water again due to the biogenic nature of BFMO. (3) Competitive oxidation and adsorption of As(III) and Sb(III) by *in-situ* generated BFMO revealed that BFMO oxidized Sb(III) more efficiently than As(III) but adsorbed As ( $0.026 \pm 0.001$  mg As/mg Fe and Mn) more efficiently than Sb ( $0.013 \pm 0.001$  mg Sb/mg Fe and Mn). The presence of Fe(II), As(III) and Sb(III) accelerated Mn(II) oxidation but inhibited the 16S rRNA gene expression level, which represents microbial activity. These data suggest that bacteria are likely to have an indirect role in Mn oxidation, that is, in the production of some substances (probably superoxides) that catalyze the Mn(II) oxidation.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jes.2016.05.026>.

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