



Antimony isotopic fractionation during Sb(III) oxidation to Sb(V): Biotic and abiotic processes

Colin Ferrari, Eléonore Resongles, Marina Héry, Angélique Désoeuvre, Rémi Freydier, Sophie Delpoux, Odile Bruneel, Corinne Casiot

► To cite this version:

Colin Ferrari, Eléonore Resongles, Marina Héry, Angélique Désoeuvre, Rémi Freydier, et al.. Antimony isotopic fractionation during Sb(III) oxidation to Sb(V): Biotic and abiotic processes. Chemical Geology, 2023, 641, pp.121788. 10.1016/j.chemgeo.2023.121788 . hal-04267874

HAL Id: hal-04267874

<https://hal.science/hal-04267874>

Submitted on 2 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

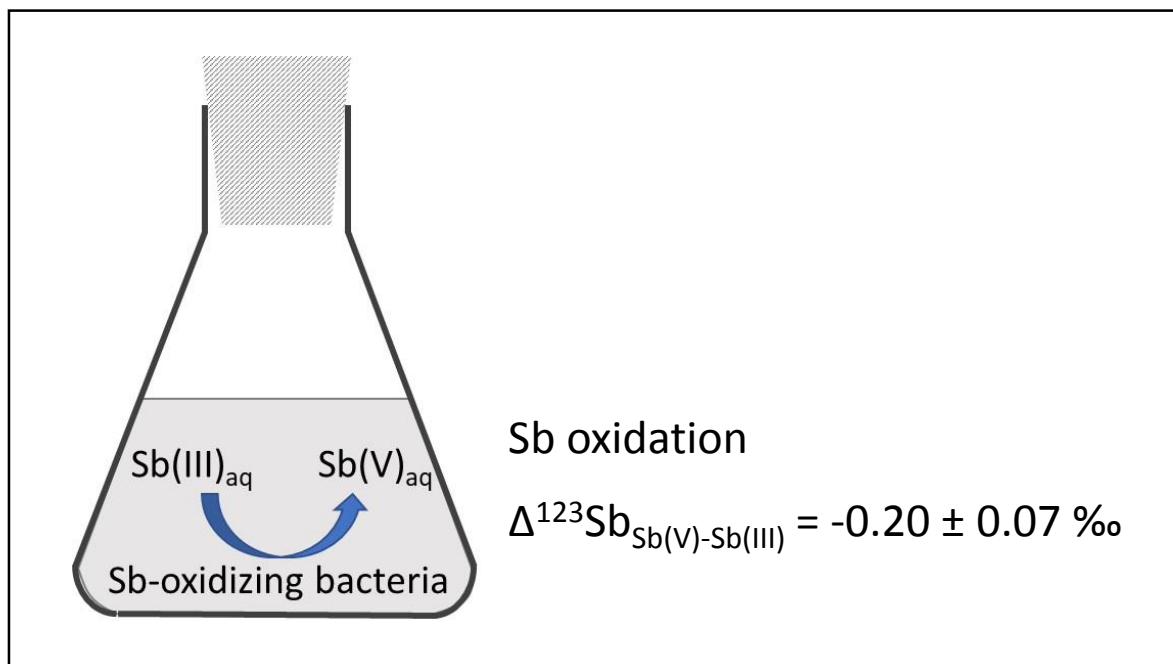
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Chemical Geology

Antimony isotopic fractionation during Sb(III) oxidation to Sb(V): biotic and abiotic processes --Manuscript Draft--

Manuscript Number:	CHEMGE16198R1
Article Type:	Research paper
Keywords:	Antimony, isotopes, oxidation, bacteria, fractionation
Corresponding Author:	Corinne Casiot HydroSciences Montpellier FRANCE
First Author:	Colin Ferrari
Order of Authors:	Colin Ferrari
	Eléonore Resongles
	Marina Héry
	Angélique Desoeuvre
	Rémi Freydier
	Sophie Delpoux
	Odile Bruneel
	Corinne Casiot
Abstract:	<p>Oxidation of antimonite (Sb(III)) to antimonate (Sb(V)) plays an important role in the control of Sb mobility in aquatic systems. Fractionation of Sb isotopes ($^{123}\text{Sb}/^{121}\text{Sb}$) during Sb(III) oxidation has been investigated in the present study at concentrations relevant to mining environments. The isotopic composition ($\delta^{123}\text{Sb}$) of dissolved Sb(III) and Sb(V) species was analysed during biotic oxidation of Sb(III) at pH 6 and ~0.1 mM Sb. Biotic experiments used a aioA gene carrier bacterial strain of the genus Ensifer isolated from Sb-rich river sediments. Chemical oxidation experiments with H_2O_2 were also conducted either in NaNO_3 or HCl medium. During biotic oxidation, the Sb(V) produced was enriched in the light isotope compared to Sb(III), with an apparent fractionation factor $\Delta^{123}\text{SbSb(V)}\text{-Sb(III)}$ of $-0.20 \pm 0.07 \text{ ‰}$. The $\Delta^{123}\text{Sb}$ value was independent of the oxidation kinetics within the range 0.03 to 0.05 $\mu\text{mol.L}^{-1}\text{.min}^{-1}$; the fractionation observed was rather attributed to kinetic effect that weakens over time in the experiments, although this hypothesis would require further investigation. During abiotic experiments in NaNO_3 medium, the Sb(V) produced was not isotopically fractionated relatively to Sb(III), while in HCl medium, the Sb(V) produced was enriched with the heavier isotope relatively to Sb(III), with a Rayleigh fractionation factor $\epsilon^{123}\text{Sb}$ of $+0.30 \pm 0.05 \text{ ‰}$. These differences were attributed to different reaction pathways involving multi-step reactions and either hydroxy- or chloride-Sb species. Altogether, these results showed a low fractionation of Sb isotopes during Sb(III) oxidation, although significant variability occurred according to experimental conditions. Further experimental research and confrontation with natural systems is necessary before applying Sb isotopes as process tracer in water.</p>

Graphical abstract



Antimony isotopic fractionation during Sb(III) oxidation to Sb(V): biotic and abiotic processes

Colin Ferrari^a, Eléonore Resongles^a, Marina Héry^a, Angélique Désoeuvre^a, Rémi Freydier^a, Sophie Delpoux^a, Odile Bruneel^a, Corinne Casiot^{a*}

^aHydroSciences Montpellier, Univ. Montpellier, CNRS, IRD, Montpellier, France

*Corresponding author

Mailing address : HydroSciences Montpellier UMR 5151
Faculté des Sciences Pharmaceutiques et Biologiques
Université de Montpellier, Bât. Hydropolis
15, avenue Charles Flahault
34093 Montpellier cedex 05, France
Email : corinne.casiot-marouani@umontpellier.fr

ORCID : 0000-0003-3318-5876

Abstract

Oxidation of antimonite (Sb(III)) to antimonate (Sb(V)) plays an important role in the control of Sb mobility in aquatic systems. Fractionation of Sb isotopes ($^{123}\text{Sb}/^{121}\text{Sb}$) during Sb(III) oxidation has been investigated in the present study at concentrations relevant to mining environments. The isotopic composition ($\delta^{123}\text{Sb}$) of dissolved Sb(III) and Sb(V) species was analysed during biotic oxidation of Sb(III) at pH 6 and ~ 0.1 mM Sb. Biotic experiments used a *aioA* gene carrier bacterial strain of the genus *Ensifer* isolated from Sb-rich river sediments. Chemical oxidation experiments with H_2O_2 were also conducted either in NaNO_3 or HCl medium. During biotic oxidation, the Sb(V) produced was enriched in the light isotope compared to Sb(III), with an apparent fractionation factor $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$ of -0.20 ± 0.07 ‰. The $\Delta^{123}\text{Sb}$ value was independent of the oxidation kinetics within the range 0.03 to 0.05 $\mu\text{mol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$; the fractionation observed was rather attributed to kinetic effect that weakens over time in the experiments, although this hypothesis would require further investigation. During abiotic experiments in NaNO_3 medium, the Sb(V) produced was not isotopically fractionated relatively to Sb(III), while in HCl medium, the Sb(V) produced was enriched with the heavier isotope relatively to Sb(III), with a Rayleigh fractionation factor $\epsilon^{123}\text{Sb}$ of $+0.30 \pm 0.05$ ‰. These differences were attributed to different reaction pathways involving multi-step reactions and either hydroxy- or chloride-Sb species. Altogether, these results showed a low fractionation of Sb isotopes during Sb(III) oxidation, although significant variability occurred according to experimental conditions. Further experimental research and confrontation with natural systems is necessary before applying Sb isotopes as process tracer in water.

Keywords

Antimony, isotopes, oxidation, bacteria, fractionation

1. Introduction

Antimony is a contaminant of emerging concern. Environmental anthropogenic contamination has been evidenced on a global scale (Krachler *et al.*, 2005; Hong *et al.*, 2012). At the Earth's surface,

anthropogenic fluxes of Sb largely exceed natural fluxes ; they represent 80 % of total Sb flux, with mining being the main contributing sector (Sen and Peucker-Ehrenbrink, 2012). Consequently, in mining-impacted streams, Sb concentration can reach extreme values of ~0.1 mM (Zhu *et al.*, 2009; Goix *et al.*, 2011). Dispersion of antimony downstream from mines is partly controlled by redox processes. Antimony is present mainly under the reduced form (trivalent form Sb(III)) in sulphide minerals (Vink, 1996), the most common being stibnite (Sb_2S_3). It is released into water upon sulphide mineral dissolution in the form of the uncharged Sb(III) hydroxide species ($\text{Sb}(\text{OH})_3$) and tends generally to be easily oxidized into Sb(V) ($\text{Sb}(\text{OH})_6^-$), even in relatively low Eh conditions (Filella *et al.*, 2001, 2002; Mitsunobu *et al.*, 2006). This oxidation process controls Sb mobility; indeed, Sb(III) is more efficiently retained onto suspended particulate matter and sediments than Sb(V) and can precipitate with iron and other various oxides and hydroxides at high Sb concentration (Roper *et al.*, 2012; Johnston *et al.*, 2020). Sb(V) can combine with iron and oxygen to form tripuhyite, an efficient Sb sink in mine waters (Leverett *et al.*, 2012), but it is mobile in the absence of iron (Resongles *et al.*, 2013). The abiotic oxidation of Sb(III) by dissolved O_2 or H_2O_2 at environmentally relevant concentrations and acid or neutral pH is very slow (e.g., the half-life of Sb(III) with 10^{-6} M H_2O_2 at pH 7 is ~ 3.2 years, Quentel *et al.*, 2004; Leuz *et al.*, 2006; He *et al.*, 2019). Some bacteria living in Sb-rich soils, sediments or acid mine drainage can mediate this process (Li *et al.*, 2013, 2015, 2018; Nguyen and Lee, 2015; Terry *et al.*, 2015; Nguyen *et al.*, 2017). Biotic Sb(III) oxidation occurs as a detoxification or respiration (providing energy for growth) mechanism (Filella *et al.*, 2007; Lehr *et al.*, 2007; Li *et al.*, 2013, 2015; Wang *et al.*, 2015; He *et al.*, 2019). Several oxidation paths involving Aio, Ano or ArsV oxidase (Wang *et al.*, 2015, Li *et al.*, 2015; He *et al.*, 2019; Zhang *et al.*, 2022) or extracellular superoxide (Wang *et al.*, 2022) have been identified.

Change of redox state is generally a source of high isotopic fractionation for transition metals and metalloids (Criss, 1999; Schauble, 2004; Wiederhold, 2015). The effect of oxidation on the isotopic fractionation was experimentally studied for numerous metals and metalloids (Cr, Cu, Se, Te, Hg, Tl, U...), although oxidation has been less studied than reduction (Johnson *et al.*, 2022), and there are no experimental studies on Sb. Theoretical studies predicted equilibrium isotope fractionation between oxidation states of Cr, Cu, Zn, Se, Hg, Tl, and Sb with an enrichment of heavy isotopes in the oxidized species (Li and Liu, 2011; Ducher *et al.*, 2016; Liu *et al.*, 2021; Ferrari *et al.*, 2022). Field studies also evidenced significant isotope fractionation associated to the oxidation of metals. Copper minerals that have undergone numerous oxidation/reduction cycles show very large isotopic variations ($^{65}\text{Cu}/^{63}\text{Cu}$), in the order of about 20 ‰ (Maréchal *et al.*, 1999; Larson *et al.*, 2003; Mathur *et al.*, 2005, 2009, 2010; Asael *et al.*, 2007). Upon oxidative dissolution of Cr(III)-rich minerals to $\text{Cr(VI)}_{\text{aq}}$, Gilleaudeau *et al.* (2016) noted an enrichment of the aqueous phase in heavy isotopes by up to +1.77 ‰ ($^{53}\text{Cr}/^{52}\text{Cr}$). This observation had also been made by Ellis *et al.* (2008) who quantified an isotopic fractionation of +1 ‰ upon oxidation of Cr(III)_s to $\text{Cr(VI)}_{\text{aq}}$. This process did not follow a Rayleigh or kinetic fractionation but rather an equilibrium fractionation, also observed by Wang *et al.* (2010). Johnson (2004) showed that the reduction of Se(VI) into Se(IV) and Se(IV) into Se(0) is the main process fractionating selenium isotopes. Fractionation ($^{80}\text{Se}/^{76}\text{Se}$) ranged from -7 to -12 ‰ (-3 to -5 ‰ for the biotic reaction) for the reduction of Se(VI) to Se(IV) and from -6 to -12 ‰ (-6 to -9 ‰ for the biotic reaction) for the reduction of Se(IV) to Se(0). In contrast, the reduction of Se(0) to Se(-II) and the oxidation (abiotic) of Se(IV) into Se(VI) generated little or no apparent fractionation (Fry *et al.*, 1988; Johnson, 2004).

Biological processes can modify or even reverse the fractionation observed for abiotic redox changes, forming lighter oxidized phases. For example, this is the case for copper, for which upon

oxidation of Cu sulphide in the presence of oxidizing bacteria, a fractionation factor $\Delta^{65}\text{Cu}_{\text{ox-red}}$ ranging from - 1‰ to -4.4 ‰ was observed, whereas for abiotic oxidation, a fractionation of +1 to +4 ‰ was measured (Moynier *et al.*, 2017). This corroborates previous observations by Kimball *et al.* (2009), demonstrating that the bacterial activity of *Acidithiobacillus ferrooxidans* oxidizing copper sulphide ($\text{CuS} + 2\text{O}_2 \rightarrow \text{CuSO}_4$, (Torma and Habashi, 1972)) favours light ^{63}Cu isotope ($\Delta^{65}\text{Cu}_{\text{ox-red}} = -0.57 \pm 0.14$ ‰).

Few studies have investigated isotopic fractionation during antimony redox changes. Rouxel *et al.* (2003) first showed that the reduction of Sb(V)_{aq} into $\text{Sb(III)}_{\text{aq}}$ induced a preferential enrichment of the reduced phase with light ^{121}Sb isotope of around -0.9 ‰. Tanimizu *et al.* (2011) showed that dissolved Sb(V) present in acid mine drainage (AMD) was around +0.35 ‰ heavier than the Sb(III) present in the stibnite minerals in mine wastes from which the AMD originated. However, it was not clear which of oxidation of Sb(III) into Sb(V) , dissolution of stibnite or adsorption most contributed to fractionation. Mackinney, (2016) also observed an important isotopic fractionation during the reduction of Sb(V) into Sb(III) and subsequent formation of $\text{Sb(III)}_2\text{S}_3$, with $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$ around +1.45 ‰; however, here again it was not clear which of precipitation or reduction most contributed to the apparent fractionation. To date, no work studied the isotopic fractionation of antimony during the oxidation of dissolved Sb(III) into Sb(V) in controlled laboratory experiments.

The goal of this work was to quantify the direction and amplitude of Sb fractionation during biotic and abiotic oxidation of $\text{Sb(III)}_{\text{aq}}$ into Sb(V)_{aq} in laboratory experiments. The Sb concentrations chosen for experiments (~0.1 mM) were relevant to mining environments, and a bacterial strain originating from a river sediment contaminated by a stibnite mine was used. These experiments represent a crucial step in the development of Sb isotopes as geochemical tracer tool in mining environments and in natural systems in general.

2. Material and methods

2.1 Reagents and materials

Sample preparation for analysis by ICP-MS, HG-ICP-MS and HG-MC-ICP-MS was carried out in a class 10 000 cleanroom facility. Ultrapure water (Milli-Q®, resistivity > 18.2 MΩ cm, Q-POP Element system, Millipore) was used for all experiments and reagent preparation. All consumables destined to analysis and Sb isotope separation (e.g. sample bottles, tubes, SPE cartridge, pipette tips...) were soaked in 10 % v/v analytical grade HCl or HNO_3 for 48 h and rinsed three times with ultrapure water before use. Suprapur® ≥ 30 % w/w HCl (Merck®), and Analpure 65–69 % w/w HNO_3 (Analytika®) were used for sample preparation and analysis. Other reagents and materials used in sample preparation for ICP-MS, HG-ICP-MS, HPLC-ICP-MS and HG-MC-ICP-MS analysis are presented in the following sections or described in detail in Ferrari *et al.* (2021).

2.2 Oxidation experiments

2.2.1 Biotic experiments

For biotic oxidation experiments, a bacterial strain called “RDB” isolated from a river sediment, “Ravin des Bernès”, draining an ancient Sb mine (Felgerette mine, Collet-de-Dèze, France), and containing 6942 mg.kg⁻¹ of Sb was used. The ability of this strain to oxidize Sb(III) into Sb(V) had been shown in preliminary experiments, although the nature of the process involved (energy production or

detoxification) was not clear from our culture trials on minimum media using tartrate antimony. Phylogenetic analysis indicated that strain RDB was affiliated to the *Ensifer/Sinorhizobium* group (Figure S1). It possesses the *aioA* gene encoding the large sub-unit of an arsenite oxidase which can also oxidize Sb(III). RDB was cultivated in CAsO1 medium at pH 6, supplemented with Sb(III). The sterile CAsO1 medium was prepared as described in Battaglia-Brunet *et al.* (2002), with minor modifications: the final concentration of $(\text{NH}_4)_2\text{SO}_4$ was 50 mg.L^{-1} and that of the yeast extract was 0.4 g.L^{-1} . Sb(III) was added from a 0.1 M Sb(III) solution made with potassium antimony(III) oxide tartrate trihydrate (Extrapure, Merck®). The filtered sterile Sb(III) solution was added to the cooled medium after autoclaving to a final concentration of 0.5 mM (for pre-cultures) or 0.1 mM (for biotic oxidation experiments); the exact Sb(III) concentration in the cultures at t_0 was determined by HPLC-ICP-MS. Three biotic oxidation experiments, called RDB0, RDB1-3 and RDB4 were conducted, including one (RDB1-3) in triplicate and with two types of abiotic controls (Abio1-3 and Control-1-3) in parallel. For each experiment, the bacterial suspension used as inoculum was collected from a pre-culture in exponential growth phase, centrifuged and resuspended in Sb(III)-free medium. Considering the fast kinetics (all Sb(III) oxidized within less than 80 h), and the complex logistics of sampling, separation and analysis, the inoculum size and the starting time of experiments were optimized to obtain samples covering a wide range of percentage of oxidized Sb(III). After addition of the inoculum (1 ml), the initial optical density at 600 nm was almost similar in the three experiments, ranging from 0.020 to 0.032. Experiment RDB4 was inoculated half day after experiment RDB1-3, in order to compensate for the lack of sampling during the night. Two kinds of abiotic controls were made. Abio1-3 corresponded to the culture medium inoculated with autoclaved bacterial pellet; it was conducted in triplicate. Control1-3 corresponded to the culture medium without inoculum, in triplicate. All cultures (300 ml) were performed in acid-decontaminated, autoclaved Erlenmeyer flasks and protected with a cellulose cap. They were incubated at 28 °C with shaking at 100 rpm. Samples (1 mL) were collected at different times (every hour, when possible, during the first day) for OD (600 nm) measurement (Figure S2), analysis of redox Sb speciation (Sb(III) and Sb(V) concentrations) and isotopic composition determination of aqueous Sb(III) and Sb(V). Samples for speciation and isotope analyses were filtrated ($<0.22 \mu\text{m}$) and frozen until analysis as a previous test confirmed that no Sb oxidation or precipitation occurred using this mode of conservation.

2.2.2 Chemical oxidation

Chemical oxidation was performed using hydrogen peroxide (Suprapur 30 % w/w H_2O_2 , Merck®) as oxidant. This strong oxidizing agent was chosen because abiotic oxidation by oxygen is too slow, indeed, no significant Sb(III) oxidation by O_2 was observed within 200 days in the pH range 4-10 (Quentel *et al.*, 2004; Leuz and Johnson, 2005). A first experiment was conducted with $\sim 0.1 \text{ mM}$ Sb(III) at pH 6, as in the biotic experiment. The methodology of Quentel *et al.*, 2004 and Leuz *et al.* (2006) was applied, except that the ionic strength was fixed with 0.01 M NaNO_3 rather than NaCl to avoid complexation of Sb with chloride. We could not use CAsO1 medium in abiotic experiments (for a strict comparison with biotic experiments) because the selective Sb(III) separation protocol used for biotic samples (see section 2.3) was not applicable in the presence of H_2O_2 due to a fast Sb(III) oxidation during the sample conditioning step. Alternatively, we took advantage of the selectivity of hydride generation toward the Sb(III) species to directly measure the isotope ratio $^{123}\text{Sb}/^{121}\text{Sb}$ of Sb(III) without separation step. This direct measurement was not compatible with the introduction of complex culture medium matrix. Our preliminary experiments showed that in a solution containing a mixture of Sb(III) and Sb(V) at a concentration of $2 \mu\text{g.L}^{-1}$ in 0.01 M NaNO_3 , 100 % of Sb(III) was converted into volatile

hydride, while a maximum of 10 % of Sb(V) was converted. This result was consistent with previous studies (Xi *et al.*, 2015). The first chemical oxidation experiment was carried out in duplicate and conducted as follows. 20 ml of 0.15 M H₂O₂ solution were added to 30 ml of 0.14 mM Sb(III) in 0.01 M NaNO₃ solution at pH 6 (adjusted using a 0.1 M NaOH solution) to reach final concentrations of 0.08 mM Sb(III) and 60 mM H₂O₂. Vials were immediately agitated and maintained at constant temperature (21 °C), with no direct sunlight. An aliquot was collected every 26 min, immediately diluted in 3 M HCl to reach a 2 µg.L⁻¹ concentration of Sb, and analysed immediately by HG-MC-ICP-MS. This measurement provided Sb(III) isotopic composition, together with Sb(III) concentration, using semi-quantitative analysis, by comparison with the signal intensity of the isotopic standard solution prepared at 2 µg.L⁻¹ of Sb (from the mono-elemental solution at 1000 µg mL⁻¹ of Sb in 20 % w/w HCl, batch number 24-175SBX, SPEX CertiPrep). The solution drained from the gas-liquid separator (hydride-generation module) was sequentially collected for determination of the isotopic composition of Sb(V), after reduction into Sb(III) in 0.5 % w/v potassium iodide and ascorbic acid, as described before (Ferrari *et al.*, 2021).

A second chemical oxidation experiment was carried out using on-line HG-MC-ICP-MS analysis. For this, a solution containing 10⁻⁴ mM Sb(III) in 3 M HCl was used. Hydrogen peroxide was added at a concentration of 0.05 mM at different time-intervals before analysis. The solution drained from the gas-liquid separator was sequentially collected and analysed for Sb(V) isotopic composition determination, as described previously.

2.3. Separation of Sb(III) and Sb(V) in biotic experiments

To analyse the isotope composition of Sb(III) and Sb(V) in the complex bacterial culture medium, physical separation of these two species is needed. For this, Sb(III) was selectively separated from Sb(V) on 0.2 g of thiol-functionalized mesoporous silica powder (hereafter referred to as TSP) at pH ~10, in 1 mL polypropylene SPE cartridges fitted with polyethylene frits (Supelco®). A modified elution protocol from Ferrari *et al.* (2021) was used (Table 1). Selective sorption conditions were based on sorption properties of Sb(III) and Sb(V) species on thiol-functionalized cotton fibers defined by Yu *et al.* (1983); Sb(V) is not sorbed at basic pH while Sb(III) is fully sorbed in this pH range. Samples containing around 500 ng of antimony were passed through the TSP column after pH adjustment to ~10 (using a 5.7 mM NH₄OH solution, Suprapur, Merck®). The selectivity of the procedure has been tested with pure Sb(V) or Sb(III) solutions; the results can be seen in Table S1. The selective recovery yields for Sb(V) and Sb(III) in elution steps 3 and 7 of the elution scheme were found between 92 and 104 %, respectively. In this modified protocol, the cleaning step 1 was carried out with ultrapure water rather than 6 M HCl to avoid Sb(V) reduction. A blank sample showed that despite this modification, the amount of Sb in elution step 7 was lower than 1 ng (<1% of total purified Sb). Antimony concentration was determined in elution fractions 3+4 (corresponding to Sb(V)) and 7 (corresponding to Sb(III)) by HG-ICP-MS. This allowed to calculate the proportion of Sb(III) and Sb(V) in the samples. The value was compared with the result of HPLC-ICP-MS analysis. Differences of ~3.5 % on average, was obtained between HPLC measurements and physical separations of Sb(III) and Sb(V) (Table S2).

Table 1: Elution scheme for the separation of the Sb(III) and Sb(V) species using thiol-functionalized silica powder modified from Ferrari *et al.* (2021).

Step	Volume (mL)	Eluent	Notes
1 Cleaning	10	Ultrapure water	

2	Conditioning	10	NH ₄ OH 5.7 mM	
3	Sample loading	10	NH ₄ OH 5.7 mM	Sb(V) collection (steps 3 and 4)
4	Rinsing	5	NH ₄ OH 5.7 mM	
5	Washing	5	0.5 M HCl	
6	Washing	6	2.5 M HCl	
7	Sb(III) elution	6	6 M HCl	Sb(III) collection

2.4 Analytical methods

All the analyses (HPLC-ICP-MS, HG-ICP-MS, HG-MC-ICP-MS) were performed on the AETE-ISO platform (OSU OREME, University of Montpellier) using iCAP Q ICP-MS and Neptune Plus MC-ICP-MS (Thermo Scientific®).

Monitoring of Sb(III) and Sb(V) concentrations in biotic experiments was carried out by High-Performance Liquid Chromatography coupled to ICP-MS (HPLC-ICP-MS) as described in Resongles *et al.* (2013).

HG-ICP-MS and HG-MC-ICP-MS instrument informations and measurement settings were taken from Ferrari *et al.* (2021), with slight modifications; for HG-MC-ICP-MS, short-term shifts in the Sb isotope ratio due to overpressure in the gas-liquid separator (Ferrari *et al.*, 2021) were attenuated by suppressing additional gas and adjusting sample gas for hydride generation. For better signal stability and reproducibility, the Sb concentration was adjusted at 2 µg L⁻¹ for all HG-MC-ICP-MS measurements. For the chemical oxidation experiments, the analysis time in HG-MC-ICP-MS was decreased in order to generate more measurement points during the oxidation kinetics, each measurement has been diminished from 80 cycles of 4.194 seconds (Ferrari *et al.*, 2021) to 40 cycles of 4.194 s (on samples and standards).

2.5 Nomenclature

Isotope composition is expressed as δ¹²³Sb (Equation 1) and corresponds to the average value for three independent isotopic measurements if not specified otherwise.

$$\delta^{123}\text{Sb}(\text{‰}) = \left(\frac{\left(\frac{^{123}\text{Sb}}{^{121}\text{Sb}} \right)_{\text{sample}} - \left(\frac{^{123}\text{Sb}}{^{121}\text{Sb}} \right)_{\text{mean std}}}{\left(\frac{^{123}\text{Sb}}{^{121}\text{Sb}} \right)_{\text{mean std}}} \right) \times 1000 \text{ (Eq. 1)}$$

where $\left(\frac{^{123}\text{Sb}}{^{121}\text{Sb}} \right)_{\text{mean std}}$ is the average of Sb isotope ratio of the Sb isotope standard solution measured before and after the sample (Sb 1000 µg mL⁻¹ in 20 % w/w HCl, batch number 24-175SBX, SPEX).

The apparent fractionation factor Δ¹²³Sb_{Sb(V)-Sb(III)} between the isotopic composition of Sb(III) and Sb(V) can be calculated according to Equation 2.

$$\Delta^{123}\text{Sb}_{\text{Sb(V)-Sb(III)}} = \delta^{123}\text{Sb}_{\text{Sb(V)}} - \delta^{123}\text{Sb}_{\text{Sb(III)}} \text{ (Eq. 2)}$$

Rayleigh fits of the isotopic composition $\delta^{123}\text{Sb}_{\text{Sb(III)}}$ and $\delta^{123}\text{Sb}_{\text{Sb(V)}}$ at different fractions (f) of remaining Sb(III) were utilized to determine the Rayleigh fractionation factor ($\epsilon^{123}\text{Sb}$) in permil, using Equation 3 (Wiederhold, 2015):

$$\delta^{123}\text{Sb}_{\text{Sb(III)}} \approx \delta^{123}\text{Sb}_{\text{Sb(III)0}} + \epsilon^{123}\text{Sb} \times \ln f \text{ (Eq.3)}$$

where $\delta^{123}\text{Sb}_{\text{Sb(III)0}}$ represents the $\delta^{123}\text{Sb}$ value of Sb(III) at the beginning of the experiment and f represents the fraction of remaining Sb(III).

The isotopic evolution of the cumulative Sb(V) product (Equation 4) is described by:

$$\delta^{123}\text{Sb}_{\text{Sb(V)}} = \delta^{123}\text{Sb}_{\text{Sb(III)0}} + \epsilon^{123}\text{Sb} \times \ln f - (\epsilon^{123}\text{Sb} \times \ln f)/(1-f) \text{ (Eq.4)}$$

3. Results

3.1 Biotic oxidation

3.1.1 Evolution of Sb(III) oxidation

Biotic oxidation of Sb(III) in the three experiments started after a lag period of about 20 h (Figure 1). It was decoupled from the exponential bacterial growth phase, which systematically occurred before 20 h, according to OD measurements (Figure S2). Sb(III) oxidation was completed within 51 h (in experiment RDB1-3) to 70 h (in experiment RDB0), which corresponded to oxidation rate values of $0.05 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (RDB1-3), $0.04 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (RDB4) and $0.03 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (RDB0) (Figure S3). Control and abiotic experiments showed less than 8 % of Sb(III) oxidized after 72 hours. This confirmed that Sb(III) oxidation was mediated by bacteria. The sum of Sb(III) and Sb(V) did not decrease during the experiments, showing that no precipitation of Sb occurred.

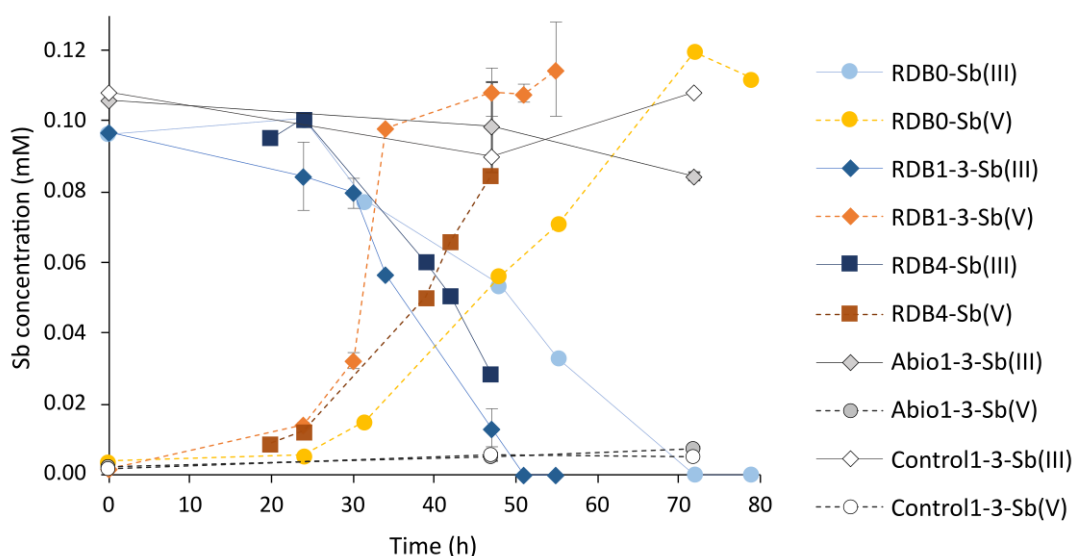


Figure 1: Evolution of the concentration of Sb(III) (solid line) and Sb(V) (broken line) in biotic (coloured symbols) experiments RDB0, RDB1-3 and RDB4 and abiotic (grey and white symbols) experiments Abio1-3 and Control1-3. Data for RDB1-3, Abio1-3 and Control1-3 are represented by the mean values of triplicate experiments and associated standard error.

3.1.2 Isotopic composition of Sb(III) and Sb(V)

The variation of $\delta^{123}\text{Sb}$ as a function of the fraction of remaining Sb(III) showed a significant fractionation of Sb isotopes between the Sb(III) species and its biotic oxidation product (Figure 2). The oxidation of Sb(III) seemed to follow a closed system equilibrium model (Figure 2), rather than a Rayleigh fractionation model (Figure S4); almost parallel increase of the isotopic composition of Sb(III) and Sb(V) occurred as the fraction of remaining Sb(III) decreased (Figure 2). The apparent fractionation factor $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$ was determined at $-0.20 \pm 0.07 \text{ ‰}$ on average, with a preferential enrichment in light isotope in the product Sb(V). The same trend was observed for the three experiments; there was no significant influence of the oxidation kinetics on the fractionation factor $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$, which was similar for RDB0 ($\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}} = -0.28 \pm 0.08 \text{ ‰}$), RDB1-3 ($\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}} = -0.25 \pm 0.04 \text{ ‰}$) and RDB4 ($\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}} = -0.18 \pm 0.05 \text{ ‰}$). It is noteworthy that in the experiment RDB1-3, the apparent fractionation is slightly higher during the first stage of the reaction ($f > 0.6$, $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}} = -0.26 \pm 0.04 \text{ ‰}$) compared to the final stage of the reaction ($f < 0.2$, $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}} = -0.20 \pm 0.04 \text{ ‰}$).

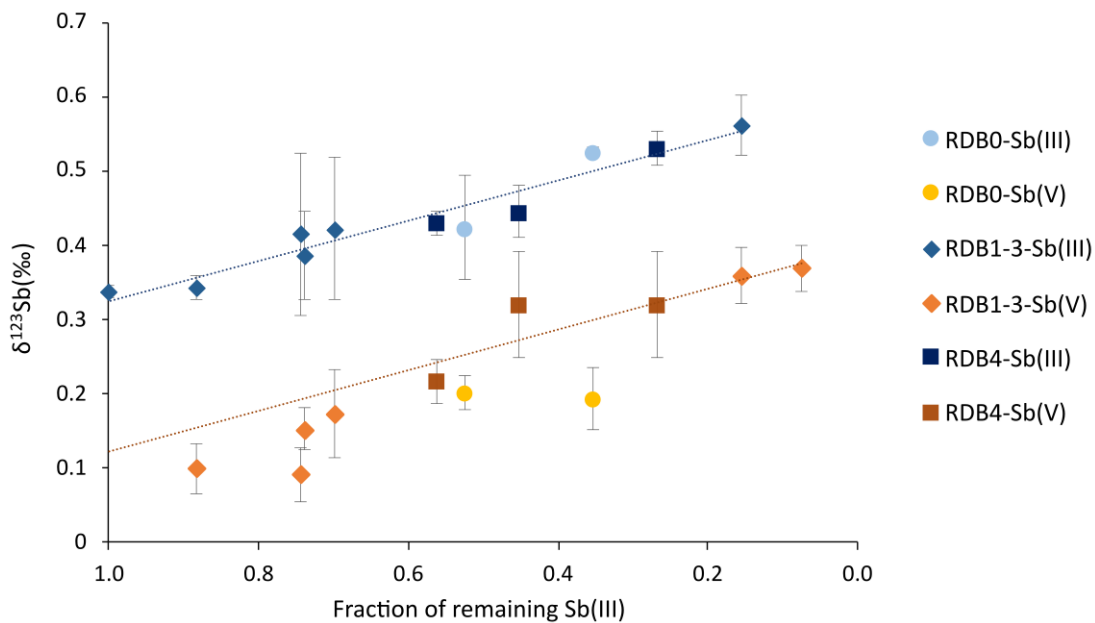


Figure 2: Isotopic composition ($\delta^{123}\text{Sb}$) of Sb(III) (blue tones) and Sb(V) (yellow-orange) during biotic oxidation as a function of fraction of remaining Sb(III) in solution. The dashed parallel lines correspond to an equilibrium isotope fractionation with the average fractionation factor $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$ of $-0.20 \pm 0.07 \text{ ‰}$.

3.2 Chemical oxidation with H_2O_2

Chemical Sb(III) oxidation with $\sim 0.1 \text{ mM}$ Sb(III) in NaNO_3 medium reached 51 % after 112 minutes (Figure 3a), corresponding to $0.3 \text{ } \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (Figure S3). The initial isotopic composition of Sb(III) ($\delta^{123}\text{Sb} = 0.30 \pm 0.04 \text{ ‰}$) was identical to that measured in the biotic experiments at t_0 ($\delta^{123}\text{Sb} = 0.33 \pm 0.04 \text{ ‰}$). The experiment did not show significant fractionation during the oxidation reaction progress between remaining Sb(III) ($\delta^{123}\text{Sb} = 0.33 \pm 0.04 \text{ ‰}$) and produced Sb(V) ($\delta^{123}\text{Sb} = 0.29 \pm 0.02 \text{ ‰}$) (Figure 3b). Similar results were obtained in a preliminary chemical oxidation experiment in which a physical separation of Sb(III) and Sb(V) with TSP had been done prior to Sb isotope measurement (Table S3).

Chemical Sb(III) oxidation with 10^{-4} mM Sb(III) in 3 M HCl showed similar proportion of Sb(III) oxidized, with 39 % Sb(III) oxidized within 131 min (Figure 3c) and $0.0004 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (Figure S3). After 190 min, Sb(III) oxidation slowed down, and there was still 16 % of Sb(III) remaining after 15 days (Figure 3c). The isotopic composition of Sb(III) initially present in the HCl 3 M solution averaged 0.40 ± 0.02 ‰, Figure 3d). The Sb(V) accumulated in solution was enriched in the heavier isotope compared to remaining Sb(III) (Figure 3d). The evolution of $\delta^{123}\text{Sb}$ was better represented by a Rayleigh model, with ϵ value of 0.3 ‰, although the cumulative Sb(V) data did not perfectly matched theoretical Rayleigh curve (Figure 3d).

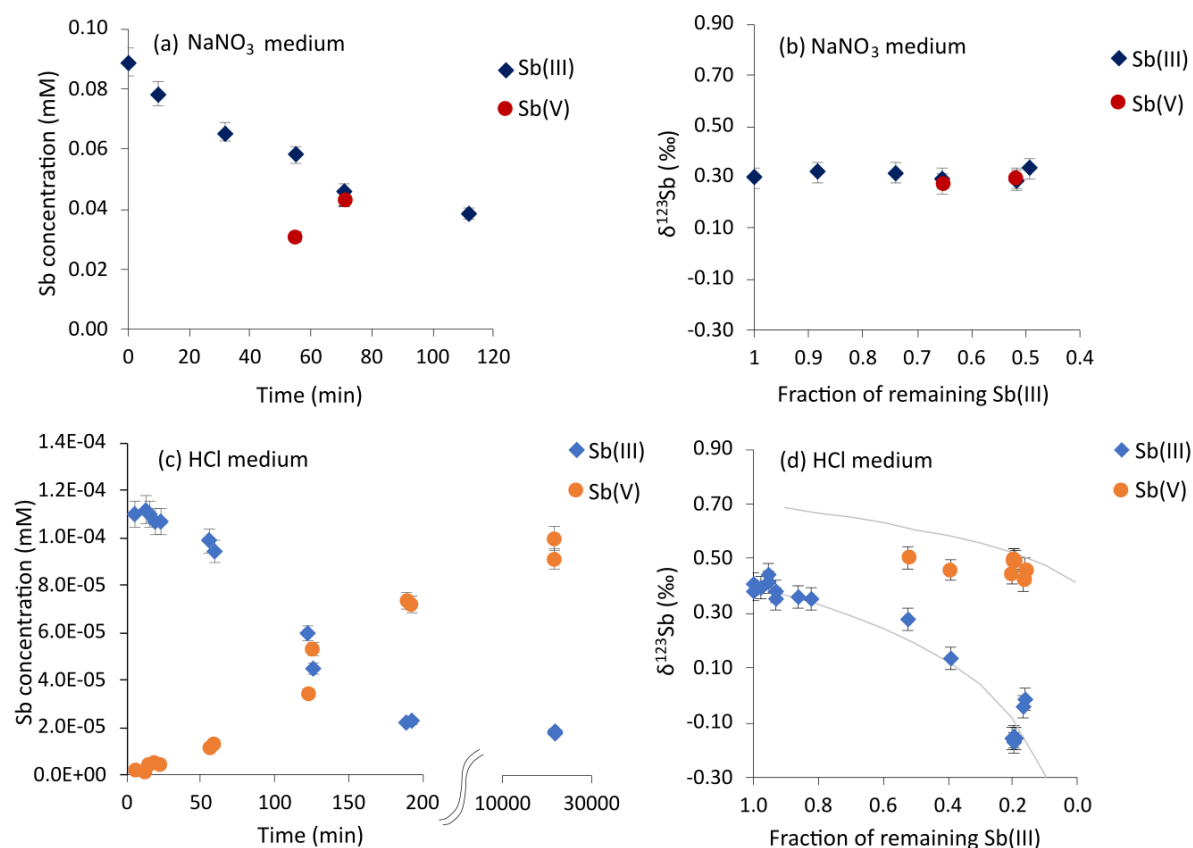


Figure 3: Evolution of the concentration of Sb(III) and Sb(V) (left) and isotopic composition $\delta^{123}\text{Sb}$ (right) in chemical oxidation experiments with ~ 0.1 mM Sb(III) in 0.01 M NaNO₃ and 60 mM H₂O₂ (a, b) and with 10^{-4} mM Sb(III) in 3 M HCl and 0.05 mM H₂O₂ (c, d). The grey lines in figure (d) represent the theoretical isotopic composition of reactant Sb(III) and cumulative product Sb(V) considering a Rayleigh fractionation model with ϵ value = 0.3 ‰. Error bars correspond to the analytical uncertainty (± 0.04 ‰) determined in Ferrari et al. (2021).

4. Discussion

4.1 Isotopic fractionation related to biotic Sb(III) oxidation

The contribution of bacterial activity on the Sb(III) oxidation rate was clearly demonstrated by the significant differences obtained between the biotic experiments and the controls without living bacteria. Biotic oxidation induced a light isotope enrichment of the Sb(V) product compared to the Sb(III) reactant. For the three biotic experiments, the evolution of $\delta^{123}\text{Sb}$ for the remaining Sb(III) and

the produced Sb(V) was best fitted with an equilibrium fractionation model (Figure 2) rather than Rayleigh model (Figure S4), and the slightly different kinetics between experiments RDB0, RDB1-3 and RDB4 did not produce significantly different fractionation factors $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$ (Figure 2). This suggests that the isotopic fractionation does not depend on the kinetics of biotic Sb(III) oxidation in the conditions of this study. This might be related to the rather slow kinetics of the biotic oxidation experiments compared to chemical oxidation experiments (one order of magnitude difference). However, there is a body of evidence that lean against an equilibrium fractionation.

First of all, there are several points in Figure 2 that do not fit the equilibrium model within the given measurement uncertainties. For example, the earliest three Sb(V) points are all significantly below the best-fit model.

Considering the enzymatic-catalyzed character of the biotic oxidation process, an isotopic fractionation of kinetic origin was expected. Indeed, biological processes are kinetically controlled (since they involve bond-breaking in enzymatic reactions) and may thus induce significant kinetic isotope effects. The bacterial strain used in our study possess the *aioA* gene involved in Sb(III) oxidation. Therefore, the involvement of an enzyme in the oxidation reaction was expected to influence the reaction pathway, and thus the isotope distribution between the reactant and the product, which can lead to kinetic isotopic fractionation.

Bacterial-catalyzed reduction reactions for elements such as Cr and Se, whose behavior is close to that of antimony showed significant kinetic isotope fractionation. Bacterial reduction of selenium oxyanions generated kinetic isotope fractionation by 3-12 ‰ (Johnson, 2004; Schilling *et al.*, 2020). For biological chromium oxyanions reduction, fractionation was 0.4-7.6 ‰ (Zhang *et al.*, 2018). Both elements showed enrichment in the light isotope in the product during these experiments. An important difference with the present study is that the processes involved during Se and Cr oxyanions reduction goes through unidirectional phase changes from dissolved to solid, without back-reaction. Such unidirectional processes were prone to imprint significant isotope fractionation, which contrasted with the present study where reactants and products remained in the aqueous phase. Furthermore, the biologically-catalyzed reaction pathway may have an important influence on the isotope fractionation. Schilling *et al.* (2020) have shown that different strains or incubation conditions produced different fractionation factors during biological selenium reduction, in relation with the reduction pathway, and the amplitude of fractionation is controlled by the rate-limiting step. Several oxidation pathways, related to different oxidases, have been identified among Sb-oxidizing bacteria. The AioA protein is a periplasmic or intermembrane enzyme (in the inner membrane) (Mukhopadhyay *et al.*, 2002; Santini and vanden Hoven, 2004); it has the capacity to oxidize Sb(III) aerobically, without the need for Sb(III) to enter the cell cytoplasm. In the present study, the location of the AioA enzyme in the periplasm may imply diffusion of Sb(III) in the inter-membrane space prior to oxidation. This may be the rate-limiting step of the whole oxidation process. Considering that diffusion produces smaller isotope fractionation than oxidation (Rodushkin *et al.*, 2004), the whole reaction path in our biological oxidation experiments may generate small kinetic fractionation amplitude.

If equilibrium fractionation is considered in the present study, the direction of fractionation, with isotopically light Sb(V) relative to the Sb(III), contradicts well-known theoretical considerations. The higher valence species of many redox-sensitive elements have shorter, stiffer, higher energy bonds than the lower valence species ; thus the heavier isotopes are enriched in the higher valence species if

equilibrium is achieved (Stephens *et al.*, 2021). Thermodynamic calculations by Ferrari *et al.* (2022) showed this is true for Sb as well. Antimony(V)-oxides minerals are preferentially enriched in the heavy ^{123}Sb isotope than Sb(III)-oxides; the equilibrium fractionation factor $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$ is about +1.2 ‰ between senarmontite ($\text{Sb(III)}_2\text{O}_3$) and Sb(V) oxide $\text{Sb(V)}_2\text{O}_5$ (Ferrari *et al.*, 2022). In the present biotic experiments, the redox and ionic structure change from a tetragonal pyramid in $\text{Sb(OH)}_{3,\text{aq}}$ to octahedral configuration in $\text{Sb(OH)}_{6,\text{aq}}^-$ was expected to produce important Sb isotope fractionation, and enrichment in the heavier isotope in Sb(V). A small fractionation in opposite direction suggests another or a masking mechanism.

It is commonly observed that bacteria synthesize organic compounds that can complex metals and metalloids. Sb(III) and Sb(V) species form stable complexes with a variety of small organic acids (Filella and May, 2005; Tella and Pokrovski, 2009). One can suggest that complexation of Sb(III) and Sb(V) with organic compounds released by bacteria fractionated Sb isotopes in the opposite direction than oxidation. Preferentially light Zn, Cd, and Hg isotopes bind to thiol groups (Wiederhold, 2015; Wigganhauser *et al.*, 2022) or carboxylic groups (for Cd, Ratié *et al.* (2021)) at equilibrium. However, it seems quite unlikely that the expected equilibrium fractionation of more than 1 per mil between Sb(V) and Sb(III), with Sb(V) enriched in heavier isotopes, could be completely reversed by complexation with organic ligands. Furthermore, considering the greater tendency of Sb(III) to form Sb-S bonds, compared to Sb(V), the fractionation between Sb(V) and Sb(III) complexed with organic thiol groups at equilibrium would increase.

Alternatively, the observed pattern in the data could be the result of a kinetic isotope effect that decreases in magnitude during the experiment. Variation of isotopic fractionation can be hypothesized because the medium where bacteria evolve is subjected to changes such as variations in the physico-chemical conditions and medium toxicity, evolution in the physiological state of the microbes as the experiments proceed and the Sb(V) and Sb(III) concentrations change. Such a change in the isotopic fractionation has been observed during microbial Se reduction experiments; as the Se species concentrations changed, fractionation increased (Herbel *et al.*, 2000). This latter pattern was attributed to enzymatic Se reduction steps becoming rate limiting upon non-respiratory pathways such as Se transport across the cell membrane. Altogether, the present data suggest a kinetic effect explaining variation of the isotopic composition over time. Further research is needed to validate this hypothesis.

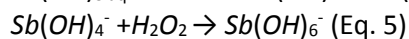
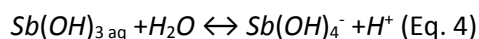
4.2 Isotopic fractionation related to chemical Sb(III) oxidation with H_2O_2

The Sb(III) chemical oxidation experiments showed little or no isotopic fractionation, with a maximum enrichment of + 0.3 ‰ in heavy isotope in the Sb(V) product. The low fractionation reported here differed from previous experimental studies dealing with Sb redox transformations. Rouxel *et al.* (2003) reported a shift of $\Delta^{123}\text{Sb}_{\text{Sb(III)}-\text{Sb(V)}}$ around -0.55 to -0.9 ‰ during abiotic Sb(V) reduction by iodide in HCl medium, in relation with a kinetic isotope effect. Mackinney (2016) showed a higher kinetic isotope fractionation of -1.42 ‰ during Sb(V) reduction by sulfide. There was no experiment reporting Sb isotope fractionation during oxidation reaction in the literature.

Comparison with other redox-sensitive elements such as selenium and chromium may help to interpret the results since these elements share common geochemical properties with Sb. Johnson (2004) showed no measurable Se isotope fractionation during oxidation of dissolved Se(IV) into Se(VI) with H_2O_2 in strongly basic solution. Regarding chromium, Zink *et al.* (2010) showed small (+0.2 ‰) isotopic fractionation during oxidation of dissolved Cr(III) to dissolved Cr(VI) in alkaline media, using

H₂O₂ as the oxidant; the reaction favoured heavy Cr isotopes in Cr(VI). These results were in line with our Sb(III) chemical oxidation experiments with 10⁻⁴ mM Sb(III) in HCl medium that showed a slight enrichment in the heavier isotope in the Sb(V) product ($\epsilon^{123}\text{Sb} = +0.3\text{‰}$). Some $\delta^{123}\text{Sb}$ values for the Sb(V) product diverged from the Rayleigh model, as observed by Zink *et al.* (2010) for Cr(III) oxidation. Zink *et al.* (2010) interpreted the poor fitting of experimental data to Rayleigh-type function by the involvement of unstable reactional intermediates Cr(IV) and Cr(V) and the divergence of the process from a single, unidirectional reaction. Indeed, kinetic isotope fractionation depends on the mechanism of reaction and the rate of individual steps. In our experiments, the Sb speciation and oxidation mechanism can be proposed by comparison with the study of Quentel *et al.* (2004). These authors investigated the oxidation of Sb(III) by H₂O₂ in a mildly acidic hydrochloric acid medium (0.5 M HCl). Quentel *et al.*, (2004) suggested that the reaction of Sb(III) oxidation by H₂O₂ goes through the formation of chlorine by H₂O₂, followed by the oxidation of Sb(III) by the chlorine formed. Chloro-complexes of Sb(III) (SbCl₄⁻) and Sb(V) (SbCl₆⁻) are expected in 3 M HCl medium (Bonner and Goishi, 1961). Quentel *et al.* (2004) suggested possible formation of transition species containing chloride bridges between SbCl₆⁻ and SbCl₄⁻ that would facilitate electron transfer at high HCl concentration. In our abiotic experiments, the HCl concentration is even higher than in the Quentel *et al.* (2004) experiments, thus such a mechanism might be involved. Therefore, a multiple-step reaction and formation of intermediate species may control the magnitude and direction of Sb isotope fractionation in our chemical Sb(III) oxidation experiment in HCl medium.

In the NaNO₃ medium, Sb isotope fractionation during Sb(III) oxidation was not significant. The mechanism was probably completely different from that occurring in HCl medium. In NaNO₃ medium at pH 6, Sb(III) is present as Sb(OH)_{3 aq}; the undissociated Sb(OH)_{3 aq} does not react with H₂O₂, the formation of Sb(OH)₄⁻ is needed for the reaction to take place (Quentel *et al.*, 2004; Leuz *et al.*, 2005), as follows:



The reaction rates of individual steps may influence Sb isotope fractionation, the extent of which being controlled by the rate-limiting step. In NaNO₃ medium, Eq. 4 (hydrolysis) may be the rate limiting step controlling Sb isotope fractionation, thus producing low fractionation compared to Eq. 5.

These results support observations made for other isotopic systems (Cr, Se). It is generally expected that oxidation reaction produces a lower fractionation than reduction reaction (Johnson *et al.*, 2022). For reduction reactions, the valence decreases and thus the equilibrium and kinetic effects of the individual steps both work in the same direction. This leads to an isotopically light product as a rule. For oxidation reaction, the kinetic and equilibrium effects of individual reaction steps potentially oppose each other, thus, we expect the oxidation reactions to have fractionations that are smaller in magnitude than reduction reactions. Indeed, certain reaction steps inside the overall process could produce isotopically heavy products (if they are close to equilibrium, with valence increasing) or could produce light products (if the forward reaction dominates for that step). Thus, the overall process, which is the combination of potentially positive and negative steps (positive epsilon values or negative values) would produce a low fractionation.

Altogether, the present data suggest that small fractionations like those seen in the present study could be the rule for Sb oxidation.

4.3 Environmental implications

Our observations have implications for the interpretation of antimony isotopic composition in aquatic environments highly enriched with Sb. The fractionation produced during biological oxidation is in the same direction than that produced by sorption of Sb(V) onto iron minerals (Zhou *et al.*, 2023), which is another major process involved in Sb behaviour in contaminated waters. This must be considered for interpretation of Sb isotopes dynamic in the environment. Furthermore, the fractionation factors obtained in laboratory conditions with a pure bacterial strain must be confronted to the apparent fractionation factors in real natural environments where more complex bacterial populations are likely to interact with Sb. The Sb isotope fractionation may vary according to the bacterial strain, in relation with different metabolic pathways, as seen for selenium reduction (Schilling *et al.*, 2020).

Concerning abiotic oxidation, additional conditions should be investigated as various complex multi-step reaction processes can generate different apparent isotopic fractionation for a same reaction (Johnson *et al.*, 2022). In particular, considering the role of iron and manganese (oxyhydr)oxides in both redox transformations and sorption of Sb in the environment (Belzile *et al.*, 2001; Resongles *et al.*, 2013; Guo *et al.*, 2018), Sb isotope fractionation related to oxidation by these phases should be investigated. For example, Dwivedi *et al.* (2022) recently showed that oxidation of aqueous Se(IV) by Mn oxide birnessite produces Se(VI) which is enriched in the lighter isotope relative to Se(IV), attributed to a kinetic effect, with a fractionation factor of -2.33‰ . Miletto *et al.* (2021) also showed that microbial Mn(II) oxidation induces a Cr isotope fractionation of up to $+0.8 \pm 0.1\text{‰}$.

Therefore, the present results have to be considered as a first step toward a better comprehension of Sb isotopes behaviour during redox processes in water.

5. Conclusion

In this study, isotopic fractionation generated by biotic and chemical Sb(III) oxidation into Sb(V) was investigated for the first time. Important variations in Sb isotope fractionation occurred according to the experimental conditions, showing either positive or negative fractionation, but always of low amplitude. Biotic oxidation with a pure bacterial strain of the genus *Ensifer* was found to favour the light isotopes of antimony in the oxidized phase, with a $\Delta^{123}\text{Sb}_{\text{Sb(V)}-\text{Sb(III)}}$ of $-0.20 \pm 0.07\text{‰}$. This isotopic fractionation could be related to kinetic fractionation that weakens over time rather than equilibrium fractionation. This could reveal the occurrence of different steps in the oxidation of Sb(III) to Sb(V) via biotic processes, and a competition between the equilibrium and kinetic fractionation. Regarding the chemical oxidation of Sb(III) with H_2O_2 , Sb isotope fractionation reached $+0.3\text{‰}$ in HCl medium; slight enrichment in the heavy isotope in Sb(V) was attributed to a kinetic effect and multi-step reactions. Investigations should be pursued further to document Sb isotope behaviour in a wide range of environmental conditions.

6. Acknowledgements

This work received financial support from the CNRS INSU EC2CO program (Project AntiBol) and ANR-22-CE01-0016 project ANTIMONY. We thank Léa Causse for trace element analysis performed on the AETE-ISO platform, OSU OREME/University of Montpellier and David Clousier and Naoual Azhir el Yousfi for their help during the experiments.

The authors warmly thank anonymous reviewers for their very relevant remarks and suggestions.

- 481 Asael, D. *et al.* (2007) 'Copper isotope fractionation in sedimentary copper mineralization (Timna
482 Valley, Israel)', *Chemical Geology*, 243(3), pp. 238–254. :
483 <https://doi.org/10.1016/j.chemgeo.2007.06.007>.
- 484 Battaglia-Brunet, F. *et al.* (2002) 'An arsenic(III)-oxidizing bacterial population: selection,
485 characterization, and performance in reactors', *Journal of Applied Microbiology*, 93(4), pp. 656–667. :
486 <https://doi.org/10.1046/j.1365-2672.2002.01726.x>.
- 487 Belzile, N., Chen, Y.-W. and Wang, Z. (2001) 'Oxidation of antimony (III) by amorphous iron and
488 manganese oxyhydroxides', *Chemical Geology*, 174(4), pp. 379–387. : [https://doi.org/10.1016/S0009-](https://doi.org/10.1016/S0009-2541(00)00287-4)
489 2541(00)00287-4.
- 490 Bonner, N.A. and Oishi, W. (1961). Antimony in HCl Solutions. Kinetics of Complex Exchange and
491 Hydrolysis Reactions. In: Kinetics of complex exchange and hydrolysis reactions of antimony. Vol. 83,
492 pp. 85–99.
- 493 Criss, R.E. (1999) *Principles of Stable Isotope Distribution*. Oxford University Press, New York. Ducher,
494 M., Blanchard, M. and Balan, E. (2016) 'Equilibrium zinc isotope fractionation in Zn-bearing minerals
495 from first-principles calculations', *Chemical Geology*, 443, pp. 87–96. :
496 <https://doi.org/10.1016/j.chemgeo.2016.09.016>.
- 497 Dwivedi, P. *et al.* (2022) 'Oxidation of Dissolved Tetravalent Selenium by Birnessite: Se Isotope
498 Fractionation and the Effects of pH and Birnessite Structure', *Frontiers in Earth Science*, 10. :
499 <https://www.frontiersin.org/articles/10.3389/feart.2022.909900> (Accessed: 6 October 2022).
- 500 Ellis, A. *et al.* (2008) 'Environmental Cycling of Cr Using Stable Isotopes: Kinetic and Equilibrium Effects',
501 In AGU Fall Meeting Abstracts (Vol. 2008, pp. H53F–08).
- 502 Ferrari, C. *et al.* (2021) 'A single-step purification method for the precise determination of antimony
503 isotopic composition of environmental, geological and biological samples by HG-MC-ICP-MS', *Journal*
504 *of Analytical Atomic Spectrometry*, 36(Issue 4), pp. 776–785. : <https://doi.org/10.1039/D0JA00452A>.
- 505 Ferrari, C. *et al.* (2022) 'Equilibrium mass-dependent isotope fractionation of antimony between
506 stibnite and Sb secondary minerals: A first-principles study', *Chemical Geology*, p. 121115. :
507 <https://doi.org/10.1016/j.chemgeo.2022.121115>.
- 508 Filella, M., Belzile, N. and Chen, Y.-W. (2001) 'Antimony in the environment: a review focused on
509 natural waters. I. Occurrence', *Earth-Sciences Reviews*, 57, pp. 125–176.
- 510 Filella, M., Belzile, N. and Chen, Y.-W. (2002) 'Antimony in the environment: a review focused on
511 natural waters. II. Relevant solution chemistry', *Earth-Sciences Reviews*, 59, pp. 265–285.
- 512 Filella, M., Belzile, N. and Lett, M.-C. (2007) 'Antimony in the environment: a review focused on natural
513 waters. III. Microbial relevant interactions', *Earth Sciences Reviews*, 80, pp. 195–217. :
514 <https://doi.org/10.1016/j.earscirev.2006.09.003>.
- 515 Filella, M. and May, P.M. (2005) 'Critical appraisal of available thermodynamic data for the
516 complexation of antimony(III) and antimony(V) by low molecular mass organic ligands', *Journal of*
517 *Environmental Monitoring*, 7(12), pp. 1226–1237. : <https://doi.org/10.1039/B511453E>.

518 Fry, B. *et al.* (1988) 'Sulfur isotope effects associated with oxidation of sulfide by O₂ in aqueous
519 solution', *Chemical Geology: Isotope Geoscience section*, 73(3), pp. 205–210. :
520 [https://doi.org/10.1016/0168-9622\(88\)90001-2](https://doi.org/10.1016/0168-9622(88)90001-2).

521 Gilleaudeau, G.J. *et al.* (2016) 'Oxygenation of the mid-Proterozoic atmosphere: clues from chromium
522 isotopes in carbonates', *Geochemical Perspectives Letters*, 2(2), pp. 178–187. :
523 <https://doi.org/10.7185/geochemlet.1618>.

524 Goix, S. *et al.* (2011) 'Influence of source distribution and geochemical composition of aerosols on
525 children exposure in the large polymetallic mining region of the Bolivian Altiplano', *Science of The Total
526 Environment*, 412–413, pp. 170–184. : <https://doi.org/10.1016/j.scitotenv.2011.09.065>.

527 Guo, W. *et al.* (2018) 'Environmental geochemical and spatial/temporal behavior of total and
528 speciation of antimony in typical contaminated aquatic environment from Xikuangshan, China',
529 *Microchemical Journal*, 137, pp. 181–189. : <https://doi.org/10.1016/j.microc.2017.10.010>.

530 He, M. *et al.* (2019) 'Antimony speciation in the environment: Recent advances in understanding the
531 biogeochemical processes and ecological effects', *Journal of Environmental Sciences*, 75, pp. 14–39. :
532 <https://doi.org/10.1016/j.jes.2018.05.023>.

533 Herbel, M.J.; Johnson, T.M.; Oremland, R.S.; Bullen, T.D. (2000). 'Fractionation of selenium isotopes
534 during bacterial respiratory reduction of selenium oxyanions.' *Geochimica et Cosmochimica Acta*,
535 64(21), pp. 3701–3709. [https://doi.org/10.1016/S0016-7037\(00\)00456-7](https://doi.org/10.1016/S0016-7037(00)00456-7).

536 Hong, S. *et al.* (2012) 'Evidence of Global-Scale As, Mo, Sb, and Tl Atmospheric Pollution in the Antarctic
537 Snow', *Environmental Science & Technology*, 46(21), pp. 11550–11557. :
538 <https://doi.org/10.1021/es303086c>.

539 Johnson, T.M. (2004) 'A review of mass-dependent fractionation of selenium isotopes and implications
540 for other heavy stable isotopes', *Chemical Geology*, 204(3), pp. 201–214. :
541 <https://doi.org/10.1016/j.chemgeo.2003.11.015>.

542 Johnson, T.M. *et al.* (2022) 'A Review of the Development of Cr, Se, U, Sb, and Te Isotopes as Indicators
543 of Redox Reactions, Contaminant Fate, and Contaminant Transport in Aqueous Systems', in *Isotopic
544 Constraints on Earth System Processes*. American Geophysical Union (AGU), pp. 237–269. :
545 <https://doi.org/10.1002/9781119595007.ch10>.

546 Johnston, S.G. *et al.* (2020) 'Antimony and arsenic speciation, redox-cycling and contrasting mobility in
547 a mining-impacted river system', *Science of The Total Environment*, 710, p. 136354. :
548 <https://doi.org/10.1016/j.scitotenv.2019.136354>.

549 Kimball, B.E. *et al.* (2009) 'Copper isotope fractionation in acid mine drainage', *Geochimica et
550 Cosmochimica Acta*, 73(5), pp. 1247–1263. : <https://doi.org/10.1016/j.gca.2008.11.035>.

551 Krachler, M. *et al.* (2005) 'Increasing atmospheric antimony contamination in the northern
552 hemisphere: snow and ice evidence from Devon Island, Arctic Canada', *Journal of Environmental
553 Monitoring*, 7(12), pp. 1169–1176. : <https://doi.org/10.1039/B509373B>.

554 Larson, P.B. *et al.* (2003) 'Copper isotope ratios in magmatic and hydrothermal ore-forming
555 environments', *Chemical Geology*, 201(3), pp. 337–350. :
556 <https://doi.org/10.1016/j.chemgeo.2003.08.006>.

557 Lehr, C.R., Kashyap, D.R. and McDermott, T.R. (2007) 'New Insights into Microbial Oxidation of
558 Antimony and Arsenic', *Applied and Environmental Microbiology*, 73(7), pp. 2386–2389. :
559 <https://doi.org/10.1128/AEM.02789-06>.

560 Leuz, A.-K. and Johnson, C.A. (2005) 'Oxidation of Sb(III) to Sb(V) by O₂ and H₂O₂ in aqueous solutions',
561 *Geochimica et Cosmochimica Acta*, 69(5), pp. 1165–1172. : <https://doi.org/10.1016/j.gca.2004.08.019>.

562 Leuz, A.-K., Mönch, H. and Johnson, A. (2006) 'Sorption of Sb(III) and Sb(V) to Goethite: Influence on
563 Sb(III) Oxidation and Mobilization', *Environement Sciences and Technologies*, 40(23), pp. 7277–7282. :
564 <https://doi.org/10.1021/es061284b>.

565 Leverett, P. *et al.* (2012) 'Tripuhyite and schafarzikite: two of the ultimate sinks for antimony in the
566 natural environment', *Mineralogical Magazine*, 76(4), pp. 891–902. :
567 <https://doi.org/10.1180/minmag.2012.076.4.06>.

568 Li, J. *et al.* (2013) 'Phylogenetic and genome analyses of antimony-oxidizing bacteria isolated from
569 antimony mined soil', *International Biodeterioration & Biodegradation*, 76, pp. 76–80. :
570 <https://doi.org/10.1016/j.ibiod.2012.06.009>.

571 Li, J. *et al.* (2015) 'Proteomics and Genetics for Identification of a Bacterial Antimonite Oxidase in
572 *Agrobacterium tumefaciens*', *Environmental Science & Technology*, 49(10), pp. 5980–5989. :
573 <https://doi.org/10.1021/es506318b>.

574 Li, J. *et al.* (2018) 'Novel Hyper Antimony-Oxidizing Bacteria Isolated from Contaminated Mine Soils in
575 China', *Geomicrobiology Journal*, 35(8), pp. 713–720. :
576 <https://doi.org/10.1080/01490451.2018.1454556>.

577 Li, X. and Liu, Y. (2011) 'Equilibrium Se isotope fractionation parameters: A first-principles study', *Earth
578 and Planetary Science Letters*, 304(1), pp. 113–120. : <https://doi.org/10.1016/j.epsl.2011.01.022>.

579 Liu, S. *et al.* (2021) 'Equilibrium Cu isotope fractionation in copper minerals: a first-principles study',
580 *Chemical Geology*, 564, p. 120060. : <https://doi.org/10.1016/j.chemgeo.2021.120060>.

581 Mackinney, J.S. (2016) *Antimony isotopes as indicator of RedOx reactions in aqueous systems:
582 fractionation during Sb(V) reduction by sulfide and isotope exchange kinetics between dissolved Sb(III)
583 and Sb(V)*. Thesis. University of Illinois at Urbana-Champaign. :
584 <https://core.ac.uk/download/pdf/158314035.pdf>.

585 Maréchal, C.N., Télouk, P. and Albarède, F. (1999) 'Precise analysis of copper and zinc isotopic
586 compositions by plasma-source mass spectrometry', *Chemical Geology*, 156(1), pp. 251–273. :
587 [https://doi.org/10.1016/S0009-2541\(98\)00191-0](https://doi.org/10.1016/S0009-2541(98)00191-0).

588 Mathur, R. *et al.* (2005) 'Cu isotopic fractionation in the supergene environment with and without
589 bacteria', *Geochimica et Cosmochimica Acta*, 69(22), pp. 5233–5246. :
590 <https://doi.org/10.1016/j.gca.2005.06.022>.

591 Mathur, R. *et al.* (2009) 'Exploration potential of Cu isotope fractionation in porphyry copper deposits',
592 *Journal of Geochemical Exploration*, 102(1), pp. 1–6. : <https://doi.org/10.1016/j.gexplo.2008.09.004>.

593 Mathur, R. *et al.* (2010) 'Patterns in the Copper Isotope Composition of Minerals in Porphyry Copper
594 Deposits in Southwestern United States', *Economic Geology*, 105(8), pp. 1457–1467. :
595 <https://doi.org/10.2113/econgeo.105.8.1457>.

596 Miletto, M. *et al.* (2021) 'Marine microbial Mn(II) oxidation mediates Cr(III) oxidation and isotope
597 fractionation', *Geochimica et Cosmochimica Acta*, 297, pp. 101–119. :
598 <https://doi.org/10.1016/j.gca.2021.01.008>.

599 Mitsunobu, S., Harada, T. and Takahashi, Y. (2006) 'Comparison of Antimony Behavior with that of
600 Arsenic under Various Soil Redox Conditions', *Environmental Science & Technology*, 40(23), pp. 7270–
601 7276. : <https://doi.org/10.1021/es060694x>.

602 Moynier, F. *et al.* (2017) 'The Isotope Geochemistry of Zinc and Copper', *Reviews in Mineralogy and
603 Geochemistry*, 82(1), pp. 543–600. : <https://doi.org/10.2138/rmg.2017.82.13>.

604 Mukhopadhyay, R. *et al.* (2002) 'Microbial arsenic: from geocycles to genes and enzymes', *FEMS
605 Microbiology Reviews*, 26(3), pp. 311–325. : <https://doi.org/10.1111/j.1574-6976.2002.tb00617.x>.

606 Nguyen, V.K. *et al.* (2017) 'Microbial oxidation of antimonite and arsenite by bacteria isolated from
607 antimony-contaminated soils', *International Journal of Hydrogen Energy*, 42(45), pp. 27832–27842. :
608 <https://doi.org/10.1016/j.ijhydene.2017.08.056>.

609 Nguyen, V.K. and Lee, J.-U. (2015) 'Antimony-Oxidizing Bacteria Isolated from Antimony-Contaminated
610 Sediment – A Phylogenetic Study', *Geomicrobiology Journal*, 32(1), pp. 50–58. :
611 <https://doi.org/10.1080/01490451.2014.925009>.

612 Quentel, F. *et al.* (2004) 'Kinetic Studies on Sb(III) Oxidation by hydrogen Peroxide in Aqueous Solution',
613 *Environmental Science and Technology*, 38(10), pp. 2843–2848. : <https://doi.org/10.1021/es035019r>.

614 Ratie, G. *et al.* (2021) 'Cadmium isotope fractionation during complexation with humic acid',
615 *Environmental Science & technology*, 55 (11), pp.7430-7444. :
616 <https://doi.org/10.1021/acs.est.1c00646>.

617 Resongles, E. *et al.* (2013) 'Fate of Sb(V) and Sb(III) species along a gradient of pH and oxygen
618 concentration in the Canoulès mine waters (Southern France)', *Environmental Science Processes and
619 Impacts*, 15, pp. 1536–1544. : <https://doi.org/10.1039/c3em00215b>.

620 Rodushkin, I. *et al.* (2004) 'Isotopic Fractionation during Diffusion of Transition Metal Ions in Solution',
621 *Analytical Chemistry*, 76(7), pp. 2148–2151. : <https://doi.org/10.1021/ac035296g>.

622 Roper, A.J., Williams, P.A. and Filella, M. (2012) 'Secondary antimony minerals: Phases that control the
623 dispersion of antimony in the supergene zone', *Geochemistry*, 72, pp. 9–14. :
624 <https://doi.org/10.1016/j.chemer.2012.01.005>.

625 Rouxel, O., Ludden, J. and Fouquet, Y. (2003) 'Antimony isotope variations in natural systems and
626 implications for their use as geochemical tracers', *Chemical Geology*, 200, pp. 25–40. :
627 [https://doi.org/10.1016/S0009-2541\(03\)00121-9](https://doi.org/10.1016/S0009-2541(03)00121-9)

628 Santini, J.M. and vanden Hoven, R.N. (2004) 'Molybdenum-Containing Arsenite Oxidase of the
629 Chemolithoautotrophic Arsenite Oxidizer NT-26', *Journal of Bacteriology*, 186(6), pp. 1614–1619. :
630 <https://doi.org/10.1128/jb.186.6.1614-1619.2004>.

631 Schauble, E.A. (2004) 'Applying Stable Isotope Fractionation Theory to New Systems', *Reviews in
632 Mineralogy and Geochemistry*, 55(1), pp. 65–111. : <https://doi.org/10.2138/gsrmg.55.1.65>.

633 Schilling, K. *et al.* (2020) 'Mass-dependent selenium isotopic fractionation during microbial reduction
634 of seleno-oxyanions by phylogenetically diverse bacteria', *Geochimica et Cosmochimica Acta*, 276, pp.
635 274–288. : <https://doi.org/10.1016/j.gca.2020.02.036>.

636 Sen, I.S. and Peucker-Ehrenbrink, B. (2012) 'Anthropogenic Disturbance of Element Cycles at the
637 Earth's Surface', *Environmental Science & Technology*, 46(16), pp. 8601–8609. :
638 <https://doi.org/10.1021/es301261x>.

639 Stephens, J.A. *et al.* (2021) 'Use of non-traditional heavy stable isotopes in archaeological research',
640 *Journal of Archaeological Science*, 127, p. 105334. : <https://doi.org/10.1016/j.jas.2021.105334>.

641 Tanimizu, M. *et al.* (2011) 'Determination of natural isotopic variation in antimony using inductively
642 coupled plasma mass spectrometry for an uncertainty estimation of the standard atomic weight of
643 antimony', *Geochemical Journal*, 45, pp. 27–32.

644 Tella, M. and Pokrovski, G.S. (2009) 'Antimony(III) complexing with O-bearing organic ligands in
645 aqueous solution: An X-ray absorption fine structure spectroscopy and solubility study', *Geochimica et*
646 *Cosmochimica Acta*, 73(2), pp. 268–290. : <https://doi.org/10.1016/j.gca.2008.10.014>.

647 Terry, L.R. *et al.* (2015) 'Microbial Oxidation of Antimony(III) with Oxygen or Nitrate by Bacteria Isolated
648 from Contaminated Mine Sediments', *Applied and Environmental Microbiology*, 81(24), pp. 8478–
649 8488. : <https://doi.org/10.1128/AEM.01970-15>.

650 Thullner, M. *et al.* (2013) 'Influence of mass transfer on stable isotope fractionation', *Applied*
651 *Microbiology and Biotechnology*, 97(2), pp. 441–452. : <https://doi.org/10.1007/s00253-012-4537-7>.

652 Torma, A.E. and Habashi, F. (1972) 'Oxidation of copper (II) selenide by *Thiobacillus ferrooxidans*',
653 *Canadian Journal of Microbiology* [Preprint]. : <https://doi.org/10.1139/m72-278>.

654 Vink, B.W. (1996) 'Stability relations of antimony and arsenic compounds in the light of revised and
655 extended Eh-pH diagrams', *Chemical Geology*, 130(1), pp. 21–30. : [https://doi.org/10.1016/0009-](https://doi.org/10.1016/0009-2541(95)00183-2)
656 [2541\(95\)00183-2](https://doi.org/10.1016/0009-2541(95)00183-2).

657 Wang, D.T. *et al.* (2010) 'Stable Isotope Fractionation during Chromium(III) Oxidation by δ -MnO₂', In:
658 American Geophysical Union, Fall Meeting 2010, abstract id. H53F-1109.

659 Wang, Q. *et al.* (2015) 'Arsenite Oxidase Also Functions as an Antimonite Oxidase', *Applied and*
660 *Environmental Microbiology*, 81(6). : <https://doi.org/10.1128/AEM.02981-14>.

661 Wang, L., Ye, L., Yin, Z., Zhang, L., Jing, C. (2022). 'Antimonite oxidation by microbial extracellular
662 superoxide in *Pseudomonas* sp. SbB1'. *Geochimica et Cosmochimica Acta*, 316, pp. 122-134.

663 Wiederhold, J.G. (2015) 'Metal Stable Isotope Signatures as Tracers in Environmental Geochemistry',
664 *Environmental Science & Technology*, 49(5), pp. 2606–2624. : <https://doi.org/10.1021/es504683e>.

665 Wiggenhauser, M.; Moore, R.E.T.; Wang, P.; Bienert, G.P.; Laursen, K. H.; Blotvogel, S. (2022). 'Stable
666 Isotope Fractionation of Metals and Metalloids in Plants: A Review.' *Frontiers in Plant Science*.
667 13:840941. <https://doi.org/10.3389/fpls.2022.840941>.

668

669 Xi, J. *et al.* (2015) 'Comparison of masking agents for antimony speciation analysis using hydride
670 generation atomic fluorescence spectrometry', *Frontiers of Environmental Science & Engineering*, 9(6),
671 pp. 970–978. : <https://doi.org/10.1007/s11783-014-0716-3>.

- 672 Yu, M.-Q., Liu, G.-Q. and Jin, Q. (1983) 'Determination of trace arsenic, antimony, selenium and
673 tellurium in various oxidation states in water by hydride generation and atomic-absorption
674 spectrophotometry after enrichment and separation with thiol cotton', *Talanta*, 30(4), pp. 265–270.
675 [https://doi.org/10.1016/0039-9140\(83\)80060-5](https://doi.org/10.1016/0039-9140(83)80060-5).
- 676 Zhang, J. *et al.* (2022) 'Oxidation of organoarsenicals and antimonite by a novel flavin monooxygenase
677 widely present in soil bacteria', *Environmental Microbiology*, 24(2), pp. 752–761. :
678 <https://doi.org/10.1111/1462-2920.15488>.
- 679 Zhang, Y. *et al.* (2018) 'Cadmium isotopic evidence for the evolution of marine primary productivity
680 and the biological extinction event during the Permian-Triassic crisis from the Meishan section, South
681 China', *Chemical Geology*, 481, pp. 110–118. : <https://doi.org/10.1016/j.chemgeo.2018.02.005>.
- 682 Zhou, W. *et al.* (2023) 'Antimony Isotope Fractionation Revealed from EXAFS during Adsorption on Fe
683 (Oxyhydr)oxides', *Environmental Science & Technology*, 57(25), pp. 9353–9361. :
684 <https://doi.org/10.1021/acs.est.3c01906>.
- 685 Zhu, J. *et al.* (2009) 'Environmental characteristics of water near the Xikuangshan antimony mine.',
686 *Acta Scientiae Circumstantiae*, 29.
- 687 Zink, S., Schoenberg, R. and Staubwasser, M. (2010) 'Isotopic fractionation and reaction kinetics
688 between Cr(III) and Cr(VI) in aqueous media', *Geochimica et Cosmochimica Acta*, 74(20), pp. 5729–
689 5745. : <https://doi.org/10.1016/j.gca.2010.07.015>.

690

691

**Antimony isotopic fractionation during Sb(III) to Sb(V) oxidation:
biotic and abiotic perspectives**

Supplementary Information

Colin Ferrari^a, Eléonore Resongles^a, Marina Héry^a, Angélique Désoeuvre^a, Rémi Freydier^a, Sophie Delpoux^a, Odile Bruneel^a, Corinne Casiot^{a*}

^a*HydroSciences Montpellier, Univ. Montpellier, CNRS, IRD, Montpellier, France*

*Corresponding author

Mailing address : HydroSciences Montpellier UMR 5151
Faculté des Sciences Pharmaceutiques et Biologiques
Université de Montpellier, Bât. Hydropolis
15, avenue Charles Flahault
34093 Montpellier cedex 05, France
Email : corinne.casiot-marouani@umontpellier.fr

This PDF file includes: Tables S1 to S3 and Figures S1 to S4.

Additional tables:

Table S1: Recovery rate of pure Sb(III) and pure Sb(V) solutions on TSP.

Solution	Step	[Sb] loaded/eluted (ng)	Yield (%)	Total Sb recovered (%)
Pure Sb(V) solution	Initial loaded solution	Sb(V) 448		
	Sb(V) collection	408	91.1	92.7
	Sb(III) collection	7.6		
Pure Sb(III) solution	Initial loaded solution =	Sb(III) 488		
	Sb(V) collection	< QL		
	Sb(III) collection	507	103.8	103.8

Table S2: Comparison between the percentage of oxidation calculated through HPLC and recovered through the physical separation of Sb(III) and Sb(V) on TSP.

Ech	Sb(III)/Sb(V)	HPLC measurement	TSP separation	Difference (%)
		[Sb] (%)	[Sb] (%)	
RDB1 t=0	Sb(III)	98.3	98.1	0.3
	Sb(V)	1.7	1.9	
RDB1 t = 24	Sb(III)	86.6	88.6	-2.0
	Sb(V)	13.4	11.4	
RDB1 t=30h	Sb(III)	72.3	67.3	5.0
	Sb(V)	27.7	32.7	
RDB t=30h	Sb(III)	72.6	69.4	3.2
	Sb(V)	27.4	30.6	
RDB3 t=30h	Sb(III)	68.1	69.7	-1.6
	Sb(V)	31.9	30.3	
RDB4 t=39h	Sb(III)	54.5	57.2	-2.7
	Sb(V)	45.5	42.8	
RDB4 t=42h	Sb(III)	43.6	47.6	-4.0
	Sb(V)	56.4	52.4	
RDB4 t=47h	Sb(III)	25.2	33.7	-8.5
	Sb(V)	74.8	66.3	
RDB1 t=47h	Sb(III)	13.9	24.9	-11.1
	Sb(V)	86.1	75.1	
RDB3 t=47h	Sb(III)	5.7	19.1	-13.5
	Sb(V)	94.3	80.9	

Table S3: $\delta^{123}\text{Sb}$ of the Sb(III) and Sb(V) species physically separated on TSP in the same H_2O_2 , Sb and NaNO_3 concentrations than the online experiment described in 2.2.2 Chemical oxidation, but at pH 9.

	Time (min)	Oxidation (%)	$\delta^{123}\text{Sb}$ (‰)	2 sd (‰)
Sb(III)	25	10	0.34	0.01
	70	18	0.33	0.01
	140	19	0.33	0.04
Sb(V)	25	10	0.35	0.05
	70	18	0.34	0.06
	140	19	0.31	0.06

Additional figures:

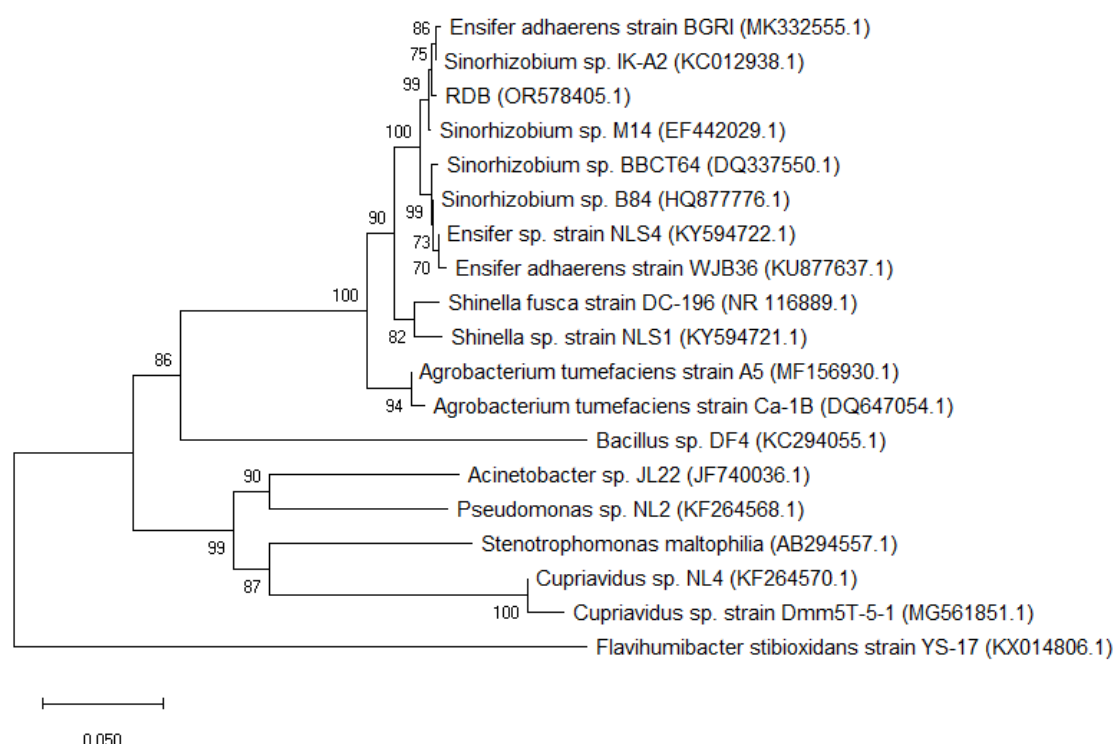


Figure S1: Phylogenetic tree constructed with the 16S rRNA sequences from RDB, its two closest relatives and other *Sb(III)* oxidizing bacteria. It was made with MEGAX (Kumar et al., 2018), using the neighbor-joining method and the Kimura correction. Nodal robustness of the tree was assessed using 1000 bootstrap replicates. DNA was extracted from RDB culture using the DNeasy PowerWater Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. 16S rRNA gene was amplified using primers 8f (Lane 1991) and 1489r (Weisburg et al., 1991) primers. Sequencing of both DNA strands was performed by GATC Biotech (Konstanz, Germany). A 1371 bp consensus sequences was obtained from the alignment of the two sequenced brands and deposited in Genbank under the accession number OR578405. References: Lane DJ (1991) rRNA sequencing. In Stachenbradt GME (ed) Nucleic acid techniques in bacterial systematics. Wiley, Chichester, United Kingdom pp115-175. Weisburg, W.G., S.M. Barns, D.A.Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplication for phylogenetic study. J. Bacteriol. 173 :697-703. Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

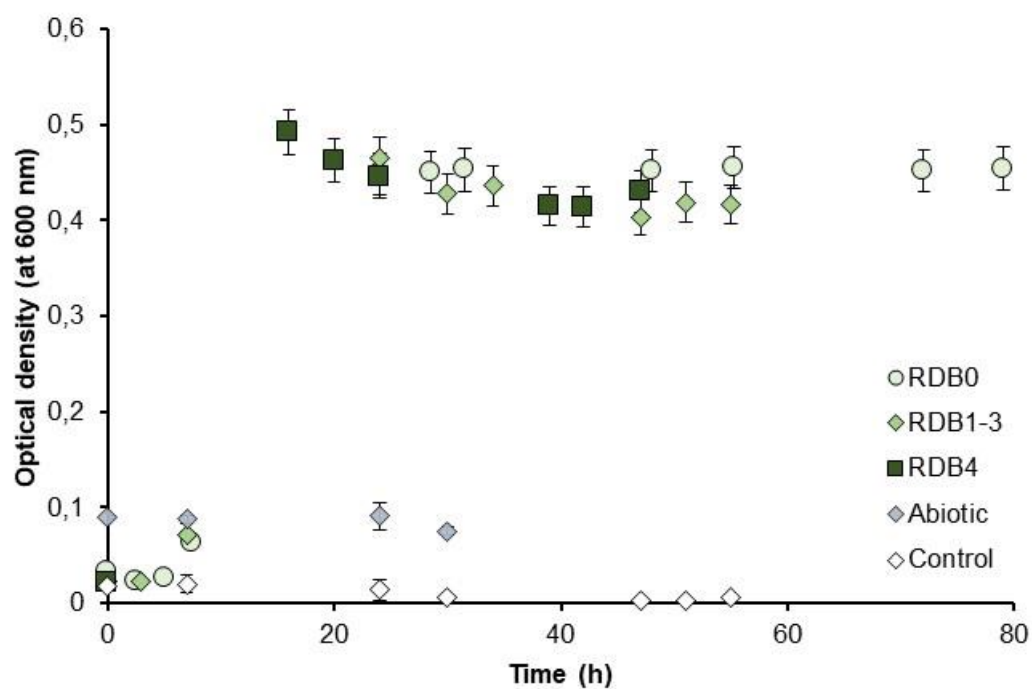


Figure S2: Optical Density measured at 600 nm for biotic (green diamonds), abiotic (light blue triangles), and control (dark blue triangles) batch.

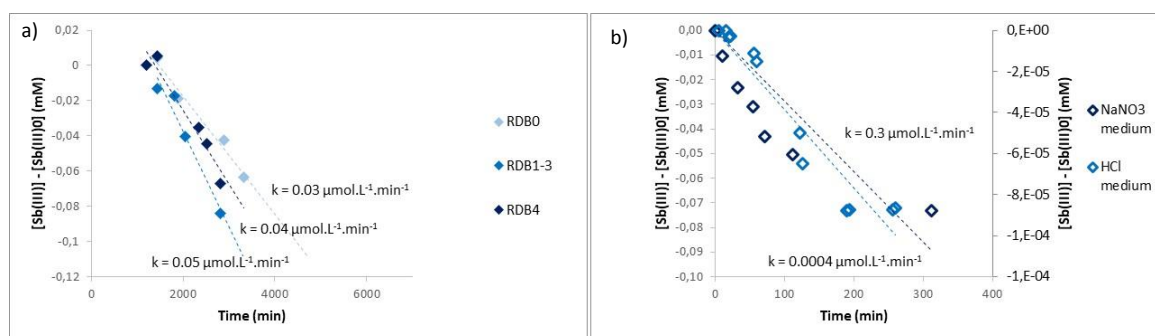


Figure S3 – Variation of Sb(III) concentration as a function of time in (a) biotic oxidation experiments exhibiting Sb(III) oxidation rate values of $0.05 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (RDB1-3), $0.04 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (RDB4) and $0.03 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (RDB0) - and (b) chemical oxidation experiments exhibiting Sb(III) oxidation rate values of $0.3 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (for NaNO_3 medium) and $0.0004 \mu\text{mol.L}^{-1}.\text{min}^{-1}$ (for HCl medium). The kinetic constant k (considering a zero-order rate law) is mentioned.

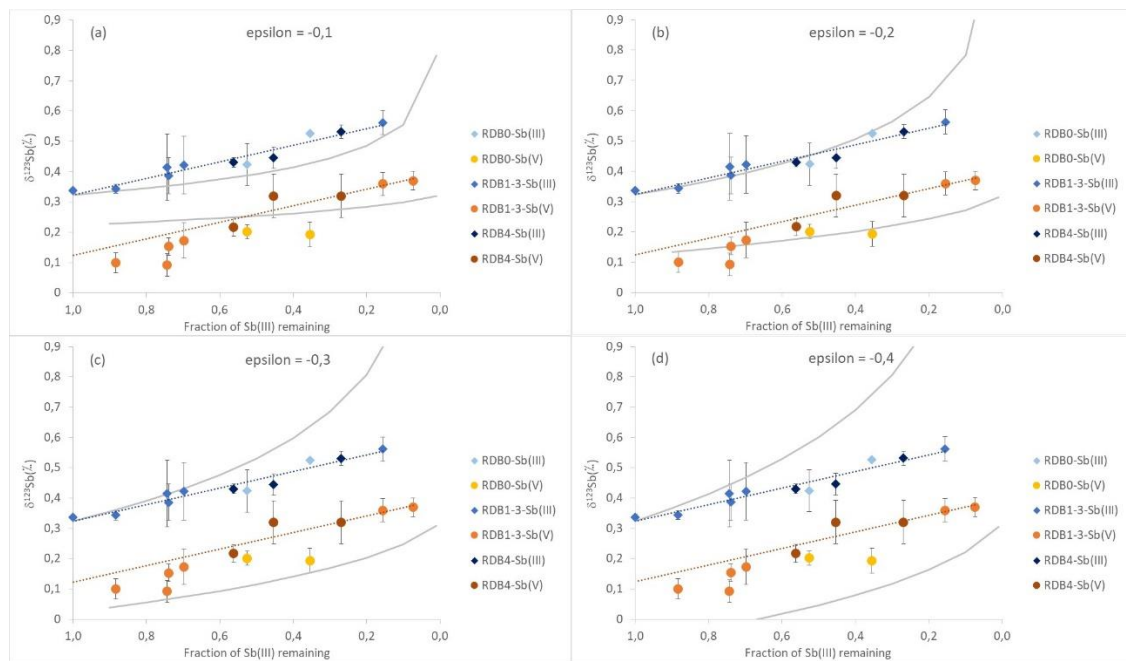


Figure S4 – Representation of Rayleigh model ($\delta^{123}\text{Sb}$ of reactant Sb(III) and cumulative product Sb(V) in grey lines) for different fractionation factors epsilon $\epsilon = -0.1$ (a), -0.2 (b), -0.3 (c) and -0.4 (d).

