Evaluating zinc isotope fractionation under sulfate reducing conditions using a flow-through cell and in situ XAS analysis

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Abstract

A flow-through cell experiment was conducted to evaluate Zn isotope fractionation during ZnS precipitation under microbially-mediated sulfate-reducing conditions. Synthetic groundwater containing 0.90 mM Zn was pumped through a cell containing creek sediment that was biostimulated to promote sulfate reducing conditions. Real-time, *in situ* X-ray absorption spectroscopy (XAS) was applied at the Zn K-edge to collect spectra *via* a Kapton window in the front of the cell over the course of the experiment. Aqueous effluent samples were collected and analysed to determine concentrations of anions and cations, and Zn isotope ratios. The flow rate was increased step-wise during the experiment to modify the residence time and produce changes in the extent of sulfate reduction, which in turn controlled the extent of ZnS precipitation. Greater enrichment in the heavier isotope in the aqueous phase relative to the input solution was associated with more extensive Zn removal. A Rayleigh curve was fit to the isotope data, where $\varepsilon = -0.27 \pm 0.06 \text{ ‰}$ (2σ). Evaluation of Zn isotope fractionation under controlled flow conditions is critical to improve the efficacy of this powerful analytical technique when applied to natural systems or remediation projects in the field.

1 Introduction

Zinc (Zn) is an essential micronutrient that is involved in numerous biogeochemical processes in the environment (Labrenz and Druschel, 2011). However, Zn can be toxic when concentrations become elevated; for example, amphipods have been shown to be impacted by Zn concentrations in the range of $200 \mu\text{g L}^{-1}$ (Besser and Leib, 2007). Anthropogenic activities such as mining can result in increased Zn concentrations in soil, surface water, and groundwater (Al et al., 1994; Moncur et al., 2005). Release of Zn from mine wastes due to sulfide mineral oxidation is frequently accompanied by elevated concentrations of sulfate (SO_4^{2-}) and either acidic or neutral pH conditions (Lindsay et al., 2009; Blowes et al., 2014). Passive treatment of Zn *via* ZnS precipitation under microbially-mediated sulfate reducing conditions has been shown to be a viable remediation technique (Waybrant et al., 1998; Blowes et al., 2000; Waybrant et al., 2002).

Under anaerobic conditions, sulfate-reducing bacteria (SRB) participate in the reduction of sulfate to sulfide, coupled with the oxidation of organic carbon (Blowes et al., 2000):

The presence of aqueous hydrogen sulfide can lead to the precipitation of Zn-sulfide, effectively removing it from solution:

Provided anaerobic conditions are maintained, this highly insoluble form of Zn (Lawrence and McCarty, 1965) can be a long-term sink.

Zinc has five stable isotopes, ^{64}Zn , ^{66}Zn , ^{67}Zn , ^{68}Zn , and ^{70}Zn , with natural abundances of 49.17 %, 27.73 %, 4.04 %, 18.45 %, and 0.61 % (Meija et al., 2016). First pioneered by Maréchal et al. (1999), Zn isotope ratio analysis has emerged as a powerful technique for

tracking geochemical processes in the environment and identifying sources of both natural and anthropogenic Zn contamination (*e.g.*, Pokrovsky et al., 2005; Borrok et al., 2009; Aranda et al., 2012). To apply Zn isotope ratio measurements as a tool to better understand complex natural systems, it is critical to characterize the Zn isotope behaviour that is attributed to each reaction mechanism. Controlled laboratory batch experiments provide insight into Zn isotope behaviour in pure systems. For example, Pokrovsky et al. (2005) observed very little fractionation during sorption of Zn to Fe and Mn oxides, reporting average shifts of up to 0.2 ‰ for $^{66}\text{Zn}/^{64}\text{Zn}$; similar ranges of fractionation were reported for Zn sorption to ferrihydrite (Veeramani et al., 2015). Juillot et al. (2008) reported $\Delta^{66/64}\text{Zn}_{\text{sorbed-aqueous}}$ values of + 0.29 ‰ for Zn sorption on goethite and + 0.53 ‰ on 2-Lines ferrihydrite, whereas Balistrieri et al. (2008) reported $\Delta^{66/64}\text{Zn}_{\text{soln-solid}}$ values of – 0.52 ‰ for Zn adsorption onto amorphous ferric oxyhydroxide. In contrast, values of $\Delta^{66/64}\text{Zn}_{\text{adsorbed-dissolved}}$ up to +2.7 ‰ were observed for Zn adsorbed to birnessite at high ionic strength (Bryan et al., 2015). Relatively large shifts in Zn isotope composition occur during mineral precipitation reactions. Aqueous Zn is enriched in the heavier isotope during ZnS precipitation, whereas during Zn-carbonate and Zn-phosphate formation, aqueous Zn is depleted in the heavier isotope (Veeramani et al., 2015). Magnitudes of Zn isotope fractionation determined experimentally are in good agreement with those predicted by computational techniques (Black et al., 2011; Fujii et al., 2011; Fujii et al., 2014). Building on these pure-system batch experiments, laboratory experiments incorporating greater complexity (*e.g.*, simulated groundwater, natural reactive materials) and saturated flow will provide an opportunity to evaluate Zn isotope ratio measurements as a tool under conditions that more closely represent real-world systems.

The objective of this study was to identify the mechanism of aqueous Zn removal under microbially-mediated sulfate reducing conditions, and to evaluate the concomitant Zn isotope behaviour during Zn removal by natural sediment under saturated flow conditions. Following the experimental design pioneered by Jamieson-Hanes et al. (2014), a flow-through cell experiment was conducted to acquire real-time, *in situ* speciation of the Zn removed from solution, and to simultaneously collect aqueous Zn for isotopic analysis. Solid-phase Zn and sulfur (S) X-ray absorption spectroscopy (XAS) was combined with aqueous pH and redox measurements, cation and anion concentrations, Zn isotopic analyses, and microbiological characterization to provide a more complete understanding of the biogeochemical processes during Zn removal by natural sediment under sulfate-reducing conditions. Assessment of Zn isotope fractionation in this system is critical for the application of isotope analyses to understand Zn cycling in natural systems, which will also aid remediation endeavours.

2 Materials and Methods

2.1 Cell design

A flow-through cell (FTC) was fabricated using high-density polyethylene (HDPE) following a similar design to that used in Jamieson-Hanes et al. (2014). The dimensions of this cell were greater than that used in a previous study, with the open-faced indentation measuring $3.8 \times 7.6 \times 1.3$ cm (37.5 mL internal volume, 28.9 cm^2 face area; Figure 1). A HDPE frame was affixed to the face of the cell to hold in place an 80 μm -thick sheet of Kapton® film allowing X-ray penetration; a rubber O-ring provided a fluid-tight seal. The outer dimensions of the cell were $17.8 \times 14.0 \times 6.4$ cm. Synthetic groundwater was passed through the cell via 2 mm ports installed at the bottom and top of the cell.

2.2 *Experimental setup*

Natural sediment was collected from the anaerobic zone of Columbia Lake in Waterloo, Ontario, Canada (43°28'30.6"N 80°33'15.0"W) to provide a natural source of microbial communities. The sediment was biostimulated in synthetic groundwater containing 3.5 mM sodium sulfate and 250 mM sodium lactate. The sediment was incubated at 25°C; the solution was replaced once a week with fresh synthetic groundwater. Within one week, the sediment was found to be reducing as indicated by formation of black iron sulfide precipitates and a hydrogen-sulfide-like smell; however, the sediment was allowed to continue to reduce for several more weeks. The biostimulated sediment was mixed with acid-washed silica sand (20 – 30 mesh) at a ratio of 40 % : 60 % by volume, to increase permeability and allow water to flow through the material. The sediment-sand mixture was packed into the FTC under a 3.5 % H₂/balance N₂ atmosphere in an anaerobic glovebox (Coy Laboratory Products Inc., Grass Lake, MI). Glass wool and Nitex mesh screen with 210 µm openings was used to prevent clogging of the influent and effluent ports.

An input solution of synthetic groundwater containing 0.90 mM Zn as ZnSO₄·7H₂O was prepared following the composition listed in Table 1. Lactate was added as the organic carbon source (250 mM as Na-lactate), while Na₂SO₄ was added to increase the initial SO₄ concentration to 4.4 mM. This solution was purged with high-purity Ar gas and equilibrated in the anaerobic glovebox. Prior to commencing the experiment, the packed FTC was saturated by using a peristaltic pump (Ismatec, Cole-Palmer, Montreal, QC) and 0.25 mm ID Pharmed pump tubing (Cole-Parmer, Vernon Hills, IL) to pump an input solution containing Zn upward through the bottom (input) port. Flow was halted after the FTC reached saturation, the input and effluent ports were closed, and the cell was allowed to sit stagnant for 72h to enhance the development of

sulfate-reducing conditions. Slow flow was applied ($1\text{--}4\text{ mL h}^{-1}$) for the following 72h. Finally, the cell was sealed, placed in an Ar-filled anaerobic canister, and transported to the synchrotron facility (stagnant for approximately 24h). Anaerobic conditions were maintained during the experiment at the synchrotron facility by mounting the cell in a He-purged plastic bag, into which the incident X-ray beam and detector were directed. Additionally, the input solution was protected with an Ar-filled Mylar balloon, while the aqueous effluent samples were collected in an Ar-purged tent (Figure 1).

Flow-through microelectrodes (Microelectrodes, Inc., Bedford, NH) measuring pH and redox conditions were installed in-line at the effluent end of the cell, and data was logged to portable Orion Star A321 meters (Thermo Scientific) at 5 minute intervals throughout the experiment. The electrodes were conditioned with 10 % HCl for approximately 10 minutes prior to calibration with pH 4, 7, and 10 buffers. The performance of the redox electrode was verified using Zobell's (Nordstrom, 1977) and Light's (Light, 1972) solutions.

To examine changes in Zn isotope behaviour, changes in the effluent Zn concentration were required. These changes were achieved by progressively increasing the flow rate step-wise from approximately 0.8 mL h^{-1} to 4.1 mL h^{-1} over the course of the experiment. The duration of the sampling interval was calculated for each flow rate to ensure sufficient volume for all required analyses (Table 2). The flow rate was increased after 12, 20, 26, 32, 36, 40, and 43 total hours of elapsed time.

2.3 *X-ray absorption spectroscopy*

Synchrotron-based X-ray absorption near edge structure (XANES) spectroscopy was performed at beamline 13-ID-E at the Advanced Photon Source (APS), Argonne National Laboratory (Argonne, IL) to acquire real-time, *in situ* Zn spectra during the experiment. Analysis

was performed at the Zn K-edge (9659 eV) in bulk mode with an unfocused incident beam ($\sim 0.5 \times 10$ mm). The incident X-ray beam intersected the Kapton® window of the cell at a 45° angle; the 4-element silicon drift fluorescence detector (Vortex ME-4, SII NanoTechnology USA Inc., Northridge, CA) was positioned at a 45° angle to the window (Figure 1). A motorized sample stage allowed for movement of the FTC during the experiment, providing the opportunity to collect spectra at multiple locations. Six locations were selected along the flow path (vertical axis) for spectroscopic analysis (Supporting Information (SI), Figure S1). Three 16-minute scans were collected at each location; each location was visited up to 10 times during the course of the experiment. The entire measurement cycle took 5 hours, and the overall experiment lasted 50 hours. The detector was moved further from the sample stage to make additional measurements at positions four and five (near the effluent end of the cell) due to detector saturation, which was observed at these locations by the end of the experiment.

Zinc reference materials were finely ground, spread on polyethylene terephthalate (PET) tape (Scotch Magic Tape, 3M, St. Paul, MN), and layered to achieve thicknesses of 300 – 500 μm . Analysis of reference materials was performed in transmission mode, including zinc oxide [ZnO], zinc sulfide [ZnS], zinc sulfate heptahydrate [ZnSO₄•7H₂O], zinc carbonate basic [(ZnCO₃)₂•(Zn(OH)₂)₃], zinc carbonate [ZnCO₃], zinc adsorbed to ferrihydrite [Zn on Fh], and zinc hydroxide [Zn(OH)₂].

Post-processing of the Zn XANES data was carried out using the program ATHENA, which is a component of the IFEFFIT software package (Ravel and Newville, 2005). To prepare the data for linear combination fitting (LCF), the three scans from each location were treated as a single time point and merged in $\mu(\text{E})$. Merging the scans reduced noise, resulting in data that was easier to fit and interpret. The scans that were merged were not found to be significantly different

from each other, though scans at a given location that were unusually noisy were excluded from the merge. Damage to the spectra due to synchrotron radiation was not observed. This process resulted in nine to ten discrete time steps for each of the six locations along the length of the cell. The merged scans were then energy shifted with respect to a Zn foil measured during the same synchrotron session before they were normalized. Linear combination fitting of the XANES region was initially performed using all available standards potentially present in the system before narrowing the scope of possibilities based on the similarity of the standards, the goodness of fit, and the probability of a given phase existing in the system. Linear combination fitting, when performed on properly normalized spectra, can provide a quantitative assessment of the species, and perhaps the phases present in the solid material, with an error of $\pm 5\%$. Sulfur XAS was subsequently used to assist in determination of the phases at each location within the cell.

After the conclusion of the experiment, the cell was maintained under anaerobic conditions and returned to the University of Waterloo. Solid materials within the cell were divided from input end to effluent end into three sections (bottom, middle, and top), divided in half, and placed in six sample bottles in an anaerobic chamber before being frozen at -80°C . Frozen samples were subsequently freeze-dried and subsampled for XANES analysis at the sulfur K-edge (2472 eV), performed at the SXRMB beamline at the Canadian Light Source (Saskatoon, SK).

Measurements at the S K-edge were made under vacuum ($< 10^{-7}$ Torr). Standards measured include elemental sulfur [S(0)], pyrrhotite [FeS], pyrite [FeS₂], zinc sulfide [ZnS], and zinc sulfate heptahydrate [ZnSO₄•7H₂O]. Elemental sulfur was measured daily to ensure the beam energies were not shifting over time, and to determine if the oxidation of sulfur would be of concern during the course of sample measurement. The dwell time for each scan was optimized

to obtain higher intensities while working within the allotted beam time, and two scans were collected for each sample.

The resulting sulfur XAS data was also processed using ATHENA (Ravel and Newville, 2005). The two scans from each sample were merged, energy shifted with respect to the most recently measured S(0) standard, then normalized. Linear combination fitting was performed using the measured standards, noting which standards could not be differentiated and which exhibited reasonably distinct spectra. Combinations were not forced to sum to one, so percentages were calculated relative to the sum of the fitted weights.

2.4 Aqueous sampling

Throughout the course of the experiment at the APS, aqueous effluent samples were collected continuously using a fraction collector (Retriever 500, Teledyne ISCO, Lincoln, NE), which was contained in an Ar-filled portable glove bag. Samples were retrieved from the fraction collector approximately every four hours to prevent significant evaporation. All samples were filtered at 0.2 μm ; odd-numbered samples were acidified to $\text{pH} < 2$ with trace-metal grade HNO_3 , while even-numbered samples were left unacidified. Samples were stored at 4°C and transported to the University of Waterloo for analysis. Cation concentrations, including Zn, were determined on acidified samples by inductively-coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific iCAP 6500) and inductively-coupled plasma mass spectrometry (ICP-MS; Thermo Scientific XSeries 2); anion concentrations were determined on unacidified samples by ion chromatography (IC; Dionex DX 600).

2.5 Microbiology

After the FTC was disassembled at the conclusion of the experiment, genomic DNA was extracted from sediment samples (1 g subsample) using Power Soil DNA Isolation Kits from MOBIO Laboratories, Inc. Extracted DNA samples were frozen and shipped to Molecular Research LP in Shallowater, TX, where 16s rRNA pyrosequencing was conducted using a Roche 454 FLX Genome Sequencer (Dowd et al., 2008).

2.6 Zn isotope analysis

Acidified aqueous samples were prepared for Zn isotope analysis following the method described in Matthies et al. (2014). Briefly, samples were combined with a ^{67}Zn - ^{70}Zn double spike solution of known isotopic composition to correct for instrumental mass bias. Following conversion to an HCl matrix, the spike-sample mixtures were loaded onto BioRad AG1-X8 anion exchange resin (200-400 mesh). Unwanted matrix elements were washed out using decreasing concentrations of HCl; Zn was eluted in the final step of the purification sequence. After conversion back to an HNO_3 matrix, the purified samples were diluted with high-resistivity (18.2 M Ω) water and prepared for isotope analysis. Each set of purifications contained two spiked aliquots of the IRMM 3702 certified isotope standard and one blank; eight of the 18 unknown samples were purified in duplicate.

Zinc isotope analysis was performed on a multi-collector ICP-MS (MC-ICP-MS; Thermo Scientific Neptune). All five Zn isotopes (^{64}Zn , ^{66}Zn , ^{68}Zn , ^{67}Zn , ^{70}Zn) were monitored simultaneously, along with ^{62}Ni and ^{72}Ge to correct for isobaric interferences. Instrumental mass bias was corrected using a double-nested iterative routine (Siebert et al., 2001), and the true isotopic composition of the samples was initially expressed as $\delta^{66}\text{Zn}$ in per mil (‰) relative to the IRMM 3702 Zn isotope standard, where:

$$\delta^{66}\text{Zn} = \left[\frac{(^{66}\text{Zn}/^{64}\text{Zn})_{\text{sample}}}{(^{66}\text{Zn}/^{64}\text{Zn})_{\text{standard}}} - 1 \right] \times 1000 \quad (3)$$

Results were normalized to daily measurements of the IRMM 3702 standards. Samples were analysed during three separate analytical events; reported results represent the average of three data points for samples purified only once, and six data points for samples purified in duplicate. Uncertainty of the data set was calculated to be ± 0.06 ‰ based on two times the root-mean-square difference of the eight samples prepared in duplicate. For the purposes of plotting and data interpretation, all effluent samples were subsequently normalized to the experimental input solution.

3 Results

3.1 Aqueous geochemistry

The pH of the input solution was determined to be 8.30 (Figure 2). At the slowest flow rate of 0.8 mL h^{-1} the effluent pH averaged 6.85 ± 0.27 (2σ). A slight decrease in effluent pH was observed as the flow rate was increased to 1.4 mL h^{-1} (6.73 ± 0.24), after which the remaining flow rates produced an average effluent pH of 6.68 ± 0.08 . Redox measurements of the effluent started at 600 mV, followed by a decrease to 340 mV at the conclusion of the experiment, suggesting a general trend toward reducing conditions (Figure 2).

Several ions, including chloride (Cl), potassium (K), and magnesium (Mg) (at higher flow rates), passed through the cell near-conservatively, deviating in the effluent very little from the input concentration (Figure 2). In contrast, calcium (Ca) and iron (Fe) were at a much higher concentration in the effluent than the input solution; this suggests that reactions occurring within the sediment released these elements into solution. Calcium was consistently observed to be at higher concentrations in the effluent than the input, with maximum concentrations of

approximately 2 mM at slower flow rates, decreasing to approximately 0.4 mM at the highest flow rate (Figure 2). This release of Ca from the reactive material may have been due to dissolution of Ca-containing minerals such as calcium carbonate. A brief but substantial release of Fe was observed at the beginning of the experiment, attaining concentrations of up to 0.17 mM in the effluent (Figure 2), suggesting reductive dissolution of Fe oxy(hydroxides) from the sediment. Nitrate was observed to decrease significantly in the effluent from the input concentration of 0.46 mM at early time, dropping below detection after the first two samples. A slight increase in nitrite was observed at early time, indicating the possible presence of nitrate reduction (Figure 2).

A slight decrease in the sulfate concentration from 4.39 mM in the influent to as low as 3.44 mM in the effluent was observed throughout the experiment; the greatest decrease in concentration occurred at the beginning of the experiment during the slowest flow rate (Figure 2). This behaviour is in agreement with the hypothesis that slow flow, and thus a greater residence time in the cell, would lead to a greater extent of sulfate reduction. A decrease in total aqueous sulfur was also observed throughout the experiment, exhibiting the lowest concentrations during the periods of slowest flow. This change in total sulfur concentrations indicates that sulfur was removed from solution, probably through sulfide mineral precipitation. It was not possible to measure aqueous sulfide concentrations to perform a mass balance calculation due to severe sample volume limitations.

3.2 Zn removal

The input Zn concentration was determined to be 0.90 ± 0.01 mM based on the average of two samples collected at the beginning and end of the experiment; the constancy of the Zn concentration indicated that no Zn precipitation occurred in the input solution over the course of

the experiment. The effluent Zn concentration remained lower than the input Zn concentration throughout the experiment, despite changes in flow rate (Figure 2). The greatest degree of Zn removal was observed at the slowest flow rate, where 72-77 % of the aqueous Zn was removed from solution. As the flow rate was increased, the degree of Zn removal decreased (*i.e.*, the effluent Zn concentration increased). At the highest flow rate, approximately 33-37 % of the aqueous Zn was removed from solution.

3.3 *In situ Zn speciation*

Experimental spectra were compared to an array of Zn standard spectra (Figure 4). Position 0 was not analyzed due to the low abundance of Zn at this location; however, visual inspection showed that scans at this location bear a slight resemblance to position 1 (Figure 3). Detector saturation at position 5 resulted in suppressed peaks (Figure 3); however, early scans at position 5 were very similar to those at position 4. Spectra from near the input end of the cell (position 1, 2, and 3) bear a resemblance to a mixture of some form of zinc carbonate, hydroxide or carbonate hydroxide, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and Zn adsorbed onto ferrihydrite (SI Figure S2). It was not possible to distinguish contributions from ZnCO_3 , $(\text{ZnCO}_3)_2 \cdot (\text{Zn}(\text{OH})_2)_3$, and $\text{Zn}(\text{OH})_2$ because these spectra are similar, and could not be differentiated (SI Figure S3). For simplicity, only the $\text{Zn}(\text{OH})_2$ spectrum has been plotted (Figure 4). The spectra from position 4 strongly resemble the ZnS standard spectrum (Figure 4). The spectra from position 3 evolved over the course of the experiment, developing features characteristic of ZnS at later time (SI Figure S2). Note that the varieties of ZnS, including wurtzite and sphalerite, were not differentiated in this study. While ZnS spectra can appear drastically different if the iron content is greater than 34 % (Ławniczak-Jabłońska et al., 1995), our ZnS standards appear very similar to other ZnS standards (Gilbert et al., 2002; Roberts et al., 2002).

3.4 Solid-phase S speciation

All three sections of the cell produced similar spectra for S K-edge XANES (Figure 5). The main difference is the height of the peak potentially associated with S(0), and the peak associated with pyrrhotite. This observation indicates that a similar composition was found throughout the cell, with more pyrrhotite near the input, and more S(0) at the effluent end of the cell.

Unfortunately, the absorbance data was not useful as a proxy for the amount of sulfur at all three locations because sample-specific optimization was performed at the time of analysis.

3.5 Microbiology

The initial lake sediment was dominated by sulfur and iron-oxidizing bacteria, including those belonging to members of *Thiobacillus*, *Sulfuricurvum*, *Sideroxydans* and *Acidovorax*, among others (Figure 6). Following biostimulation, the microbial community in the reduced sediment was dominated by members of bacteria belonging to *Desulfovibrio* and *Anaerospira* spp (Figure 6). *Desulfovibrio* is a genus of Gram negative sulfate-reducing anaerobic bacteria, commonly found in aquatic environments with high levels of organic material, as well as in water-logged soils. Species of bacteria belonging to the *Desulfovibrio* genus primarily respire sulfate and produce sulfide. However a number of species belonging to the *Desulfovibrio* genus have also been reported to reduce heavy metals such as uranium and chromium (Lovley et al., 1993; Lovley and Phillips, 1994). Species belonging to the *Anaerospira* genus are relatively rare, but have been reported to occur in coastal environments (Boehm et al., 2014). *Desulfovibrio* spp were dominant in the sediment along the length of the FTC during the experiment (Figure 6).

3.6 Zn isotope ratios

Zinc isotope measurements were performed on the aqueous Zn in both the input and effluent samples. The Zn isotope ratio of the input solution was $\delta^{66}\text{Zn} = -0.19 \pm 0.05 \text{ ‰}$ relative to the IRMM 3702 standard. To simplify the discussion of experimental results, all samples were normalized to the input solution value, resulting in a value of $\delta^{66}\text{Zn} = 0.00 \pm 0.05 \text{ ‰}$ for the input. Zinc isotope ratio values for the effluent, normalized to the input solution, are presented with effluent Zn concentrations in Figure 7, plotted over time. At early time, the effluent isotope values were enriched in the heavier isotope as compared to the input, with values up to $\delta^{66}\text{Zn} = +0.48 \pm 0.03 \text{ ‰}$. This isotope signature corresponds with the slowest flow rate, and the greatest degree of Zn removal. As the flow rate was increased and the effluent Zn concentrations increased (*i.e.*, lesser degree of Zn removal), the effluent isotope ratio values approached the input isotope composition. At the conclusion of the experiment, as the highest flow rate was achieved and less than 40 % of the aqueous Zn was being removed from solution, the isotope signature of the effluent was $\delta^{66}\text{Zn} = +0.09 \pm 0.02 \text{ ‰}$.

4 Discussion

4.1 Removal rates

Sulfate removal rates were calculated for each of the flow rates by subtracting the effluent sulfate concentration from the input concentration, and dividing the result by the residence time. Removal rates ranged from $0.03 - 0.05 \text{ mM h}^{-1}$ at the slowest flow rate (0.8 mL h^{-1}), increasing up to $0.07 - 0.08 \text{ mM h}^{-1}$ at the fastest flow rate (4.1 mL h^{-1}). These rates compare well with those calculated by Waybrant et al. (1998) for batch experiments to evaluate reactive mixtures for use in permeable reactive walls to treat mine drainage, where removal rates ranging from

0.01 mM h⁻¹ to 0.07 mM h⁻¹ were observed. Unlike the current study where Na-lactate was used as an organic carbon source, Waybrant et al. (1998) used solid organic substrates, such as sewage sludge, wood chips, and leaf mulch; the organic carbon source was attributed, in part, to the variability in the calculated sulfate removal rates. Subsequent column experiments using different mixtures of these same solid organic substrates exhibited a similar range of sulfate removal rates, with values from 0.02 mM h⁻¹ to 0.05 mM h⁻¹ (Waybrant et al., 2002). In contrast, a full-scale permeable reactive barrier installed at the Nickel Rim mine site in Ontario, Canada, designed to use bacterially-mediated sulfate reduction to promote metal-sulfide precipitation, exhibited sulfate removal rates in the range of 0.005 – 0.007 mM h⁻¹ (Benner et al., 2002). The difference between these rates and those calculated in the current study may be related to the organic carbon source used in the barrier (municipal compost), or to the lower average water temperature.

Zinc removal rates were calculated following the same approach. There was very good agreement between the Zn and sulfate removal rates, with Zn values of 0.04 – 0.05 mM h⁻¹ at the slowest flow rate (0.8 mL h⁻¹), and 0.08 – 0.09 mM h⁻¹ at the fastest flow rate (4.1 mL h⁻¹). This suggests that these constituents were monotonically removed from the system, most likely *via* the pathway of sulfate reduction followed by ZnS precipitation (Eq. 1 & 2).

An increasing trend in both the sulfate and Zn removal rates was observed in the flow-through cell experiment as the flow rate was increased. This trend could be due to a build-up of residual sulfide in the system at later times; however, the consistent 1:1 correlation between the sulfate and Zn removal rates suggest that this likely did not occur. The more likely explanation for this increasing trend is that the system was nutrient-limited at early time, during the slower flow regime. A limit in either the sulfate or the lactate may have restricted the rate of sulfate removal;

as the flow was increased, and more sulfate and lactate were supplied to the system, the rate of sulfate removal (and thus Zn removal) increased. In addition, more rapid flushing of metabolic by-products may have occurred at the faster flow rates, contributing to the increase in the sulfate removal rate. Despite the observed variation, the range of sulfate removal rates is in good agreement with published values for similar systems.

4.2 *Linear combination fitting of XAS spectra*

A comparison of the fluorescence counts at the input end of the cell to those at the effluent end indicates that only a small mass of Zn accumulated at the influent end, and a much larger mass of Zn accumulated at the effluent end (Figure 3). Linear combination fitting was used to estimate the relative abundance of phases present in each position along the cell (Figure 8; SI Figure S4). The goodness of fit is demonstrated by Figure S5 (SI), where the derivative of two representative spectra are plotted with the results of the LCF. While the fitting procedure suggested the presence of elemental Zn at all locations, this phase was excluded from the final fit due to the very low likelihood of formation in the system (Li and Zhang, 2007). The Zn XANES spectra collected at the influent end suggest that sorption or precipitation of a Zn hydroxide, Zn carbonate, or hydroxycarbonate phase was the dominant Zn removal mechanism at this location (Figure 8). However, this removal mechanism likely only played a minor role in the overall mass of Zn removed from solution (Figure 3). It is also possible that some of these Zn phases pre-existed on the sediment before the beginning of the experiment.

The Zn XANES spectra suggest that Zn accumulated at the effluent end of the cell was predominantly in the ZnS form, though a small amount of a Zn carbonate/hydroxide was initially detectable in this region (Figure 8). The S XANES spectra analysis suggests that the proportion of S in the ZnS phase is roughly identical at the three post-experiment positions (Figure 9). The

goodness of fit for the S XANES LCF is demonstrated in Figure S6 (SI). It is possible that the accumulation of these phases is entirely dependent on the amount of sulfur available, and any differences in the composition at the effluent, middle, and influent regions of the cell are related to the availability of other elements.

The greater accumulation of ZnS at the effluent end of the cell probably reflects the residence time required for sulfate reduction and ZnS precipitation. The magnitude of the ZnS signal compared to the Zn carbonate/hydroxide signal indicates that ZnS precipitation was the predominant mechanism of Zn removal over the duration of the experiment (Figure 3). The greater abundance of S(0) near the effluent end of the cell also suggests that residence time is a factor. Elemental sulfur can be produced through incomplete microbial sulfate reduction (Bishop et al., 2013), or by the oxidation of sulfides (Pyzik and Sommer, 1981; Steudel, 1996). Care was taken to preserve the samples in an anoxic environment prior to analysis, suggesting that S(0) formed *in situ*.

Although $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ may also be present according to the S XANES, it was not found during the analysis of the Zn XANES spectra. It should be noted that the $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ spectra bears a similar appearance to other SO_4 spectra, so the spectra may reflect the presence of SO_4 from the input solution, un-associated with Zn. The majority of S present in the system is associated with Fe as pyrrhotite (Figure 9). There are two possible explanations for this observation; pyrrhotite formation could arise due to the presence of Fe-sulfides in the sediment, which was collected from an anaerobic lake environment, or dissolved iron oxides re-precipitated as iron sulfides in the sulfur-reducing environment. However, due to the stoichiometric relationship between Zn and S removal rates within this system, the first hypothesis is a more likely explanation for the presence of pyrrhotite.

4.3 Zn isotope fractionation

The isotope data was fit using the Rayleigh equation (Rayleigh, 1902; Figure 10), due to the isolation of the reaction product from the reactants:

$$\frac{R}{R_0} = f^{(\alpha-1)} \quad (4)$$

where R is the $^{66}\text{Zn}/^{64}\text{Zn}$ isotope ratio, R_0 is the initial $^{66}\text{Zn}/^{64}\text{Zn}$ isotope ratio, α is the isotopic fractionation factor, and f is the fraction of Zn remaining in solution. For convenience, the extent of fractionation is expressed in terms of the isotopic fractionation value using the epsilon notation (ϵ) according to the following equation:

$$\epsilon = (\alpha - 1) \times 1000 \quad (5)$$

The uncertainty on the isotopic fractionation value was calculated as two times the standard error of the best-fit used to determine the isotopic fractionation factor, following the method of Kitchen et al. (2012). An isotopic fractionation value of $\epsilon = -0.27 \pm 0.06 \text{ ‰}$ was found to provide the best fit to the flow-through cell data (Figure 10).

During their investigation of Zn isotope variation in hydrothermal systems, Wilkinson et al. (2005) suggested that the precipitation of ZnS may involve preferential removal of the lighter isotopes from solution, resulting in a solution enriched in the heavier isotopes. Archer et al. (2004) observed a similar trend in Zn isotope fractionation during precipitation of ZnS. Theoretical calculations performed by Fujii et al. (2011) also suggested that the lighter Zn isotopes will be enriched in the sulfide species (up to $\delta^{66}\text{Zn} = -0.6 \text{ ‰}$).

The most relevant comparison for the current study comes from a series of batch experiments performed by Veeramani et al. (2015), in which Zn isotope behaviour was characterized during precipitation of ZnS, Zn-carbonate, and Zn-phosphate. Precipitation of these Zn phases was

achieved by adding varying concentrations of counteranions (Na_2S_2 , NaHCO_3 , and Na_3PO_4) to pure solutions of ZnSO_4 or ZnCl_2 , under static conditions; aqueous samples were collected after 24 hours for Zn isotope ratio analysis. Results from these batch experiments demonstrated an enrichment in the heavier isotope in the aqueous Zn during ZnS precipitation, while a depletion was observed during precipitation of carbonate and phosphate phases. The isotopic fractionation for ZnS precipitation was determined to be $\epsilon = -0.30 \pm 0.04 \text{ ‰}$; a Rayleigh curve describing this degree of fractionation is plotted along with the flow-through cell data in Figure 10 (dashed line).

The close agreement between the ϵ values for this study and ZnS precipitation from Veeramani et al. (2015) suggests that the overwhelming majority of the Zn removal from solution occurred *via* ZnS precipitation. Although the XAS data suggests the presence of a sorbed phase or a Zn carbonate or hydroxide phase near the input end of the cell, the contribution of this material to the overall mass of Zn removed from solution must have been very modest in order to have little to no effect on the bulk isotope signature.

Veeramani et al. (2015) also performed batch experiments to determine the time required to achieve isotopic equilibrium for the precipitation of each Zn phase (results included in their Supporting Information). Isotopic equilibrium was rapidly achieved for ZnS precipitation, producing a stable $\delta^{66}\text{Zn}$ value relative to the input solution within the first 15 minutes of the reaction. The shortest residence time for the flow-through cell in the current study was ~ 3.5 hours, which was observed at the fastest flow rate (4.1 mL h^{-1}). As this residence time is significantly greater than the time required for isotopic equilibrium determined by Veeramani et al. (2015), it is likely that Zn isotopic equilibrium was achieved throughout the flow-through cell experiment.

4.4 *Implications for field studies*

The application of Zn isotope ratio measurements to assist in the interpretation of measurements derived from natural systems and remediation endeavours requires an understanding of Zn isotope fractionation. Zinc removal under microbially-mediated sulfate reducing conditions is a valuable system to study as this process is widely observed in natural and remediation systems. Controlled studies such as that conducted by Veeramani et al. (2015) are critical for providing a clear characterization of Zn isotope fractionation associated with individual mechanisms. However, it is also important to evaluate more complex systems, such as the experiment conducted in this study, to extend the understanding of Zn isotope behaviour to a system where multiple geochemical processes may be involved. In addition, it is essential to consider the effect of saturated flow conditions, as flow dynamics can influence the degree of isotope fractionation (Jamieson-Hanes et al., 2012; Jamieson-Hanes et al., 2015). Results from this study demonstrate that the application of Zn isotope ratio measurements, combined with XAS and aqueous geochemical measurements, can provide valuable insight into the complex geochemical systems encountered in field studies.

The source of sulfide differed between this study and the precipitation batch experiment of Veeramani et al. (2015). Whereas the sulfide was introduced to the batch experiment abiotically with the addition of Na_2S_2 , the sulfide in the flow-through cell was produced during microbially-mediated sulfate reduction. However, the overall result was that the precipitation of ZnS in both experiments produced nearly identical Zn isotope fractionation values. In a more complex field-scale system, it may be useful to measure both Zn and S isotope ratios in the aqueous- and the solid-phase. The coupling of these isotope measurements could potentially provide insight regarding the role of abiotic vs. biologically-mediated reactions, and perhaps give more

information about the biogeochemical processes governing Zn remediation than Zn isotope ratio measurements alone are able to provide.

5 Conclusions

The purpose of this study was to characterize Zn isotope fractionation during ZnS precipitation under sulfate reducing conditions. Multiple lines of observation were used to determine that Zn removal most likely occurred *via* ZnS precipitation. Traditional aqueous geochemical parameters showed decreasing sulfate concentrations in the effluent were associated with decreasing aqueous total sulfur concentrations. Removal of Zn from solution was found to be most effective at slower flow rates, where the greater residence time led to an increased extent of sulfate reduction; a consistent 1:1 correlation between the sulfur and Zn removal rates provided further evidence of ZnS precipitation. Real-time, *in situ* XAS analysis at the Zn K-edge showed that the overwhelming majority of the Zn removed from solution was as ZnS, which was most abundant at the effluent end of the cell. The isotope composition of the aqueous Zn became more enriched relative to the input solution as a greater proportion of Zn was removed from solution, following a Rayleigh trend where $\epsilon = -0.27 \pm 0.06 \text{ ‰}$. This trend is similar to the isotope fractionation value determined for precipitation of ZnS in a batch system (Veeramani et al., 2015).

Results from this study provide valuable information on the Zn isotope behaviour under sulfate-reducing conditions. The use of a variety of analytical techniques was paramount in providing the best possible understanding of the geochemical processes within this system. Application of real-time, *in situ* XAS analysis allowed for critical evaluation of Zn treatment as the experiment progressed. Future work coupling the analysis of multiple isotope systems, such as Zn and S, may provide even further insight into the mechanisms of Zn removal. Controlled

laboratory studies such as the one described here are essential for understanding Zn isotope fractionation prior to the application of this promising new technique.

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Tables

Table 1. Composition of flow-through cell input solution.

Parameter	Value
pH	8.30
Na (mM)	272
Lactate (mM)	250
SO ₄ (mM)	4.44
Zn (mM)	0.90
Mg (mM)	0.97
K (mM)	0.42
Ca (mM)	0.07
Fe (μM)	11.76
Co (μM)	0.79
Mn (μM)	0.60
Cu (μM)	0.25
Mo (μM)	0.18
Ni (μM)	0.16
Cl (mM)	2.62
NO ₃ (mM)	0.46

Table 2. Flow-through cell experimental design.

Estimated flow rate (mL h ⁻¹)	Elapsed time at given flow rate (h)	Number of samples collected
0.8	12	4
1.4	8	3
1.9	6	3
2.5	6	4
2.7	4	3
3.3	4	4
3.8	3	3
4.1	6	8

Figures

Figure 1. Schematic of the flow-through cell setup. Input solution was pumped upward through the cell; effluent passed through in-line pH and redox microelectrodes before being collected in the fraction collector. A Kapton window allowed the passage of the X-rays at an incident angle of 45° . The detector was positioned at 90° to the beam to collect the X-ray fluorescence emitted from the sediments within the flow-through cell. The input solution, cell, and fraction collector were maintained under anaerobic conditions.

Figure 2. Aqueous geochemical data measured in the effluent over time. Input concentrations and pH are indicated by dashed lines. The flow rate was increased step-wise over time, starting at 0.8 mL h⁻¹ and ending at 4.1 mL h⁻¹.

Figure 3. Zinc XANES spectra collected at each of the six positions throughout the FTC, at the beginning of the experiment (left) and at the end of the experiment (right), plotted as X-ray absorption *vs.* energy (*i.e.*, not normalized). Position 0 is closest to the bottom of the cell (input), whereas position 5 is closest to the top (effluent). Positions 4 and 5 showed the greatest accumulation in Zn mass, as demonstrated by the large increase in X-ray absorption over time.

Figure 4. Zinc standards used for linear combination fitting (left) and normalized sample spectra from different cell positions over the course of the experiment (right). Position 1 is near the bottom of the cell, while position 4 is closer to the top. Spectra are presented for timesteps at the beginning (1), middle (5), and end (10) of the experiment.

Figure 5. Normalized sulfur XANES collected after the experiment ended on samples (right) from three sections of the solid-phase material (bottom, middle, and top). Sulfur standards used for linear combination fitting are shown on the left.

Figure 6. Microbial composition of the sediment, before and after biostimulation, and at three locations along the length of the FTC after the end of the experiment (bottom, middle, and top). Dominance of *Desulfovibrio spp.* is indicative of anoxic and sulfate-reducing conditions.

Figure 7. Effluent Zn concentrations (blue circles) and associated $\delta^{66}\text{Zn}$ values (yellow triangles) plotted over time. All isotope values were normalized to the input solution ($\delta^{66}\text{Zn} = 0.00 \pm 0.05$ ‰). Error bars represent two times the standard deviation on data derived from duplicate sample preparation (for some samples) and triplicate analysis by MC-ICP-MS (all samples). Horizontal lines indicate the input solution Zn concentration (dotted) and Zn isotope signature (dashed).

Figure 8. Linear combination fitting results for Zn K-edge spectra. The left plot depicts position 1 of the cell, while the right plot depicts position 4. Each time step was 50 minutes long, and each position was revisited every five hours.

Figure 9. Linear combination fitting results using the derivative for S K-edge XANES. Measurements were performed on subsections of the cell material at the end of the experiment.

Figure 10. Aqueous isotope results for the flow-through cell plotted as $\delta^{66}\text{Zn}$ vs. the fraction of Zn remaining in solution. The data was fitted with a Rayleigh curve, resulting in an isotopic fractionation of $\epsilon = -0.27 \pm 0.06 \text{ ‰}$ (solid line). A curve with the value $\epsilon = -0.30 \pm 0.04 \text{ ‰}$ is plotted to represent the isotopic fractionation determined for ZnS precipitation by Veeramani et al. (2015) (dashed line).