ISOTOPIC STUDY OF URANIUM: DETERMINING THE ISOTOPIC FRACTIONATION OF URANIUM DURING ABIOTIC REDUCTION WITH IRON(II)

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Geology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2014

Urbana, Illinois

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Abstract

Uranium is a contaminant of interest due to its toxicity and mobility in groundwater. It is a redox active element in which the oxidation state can change by the addition or removal of electrons. In one method of groundwater remediation, mobile, hexavalent uranium (U(VI)) can be reduced to immobile, tetravalent uranium (U(IV)) by microbial or abiotic chemical reactions. Unfortunately, due to uranium's complex geochemistry, measuring concentrations does not reliably give an accurate indicator of the efficiency of removal. However, uranium isotope ratios can provide a direct way to track the extent of reduction.

At the Old Rifle site (Colorado, USA), reduction of U(VI) to U(IV) was induced via injections of acetate to stimulate microbial activity. A decrease of ~1‰ in the $^{238}U/^{235}U$ ratio of the dissolved U(VI) was observed and correlated closely to the decrease in concentration (Bopp et al., 2010). However, the primary mechanism of chemical reduction, microbial or abiotic, is still not well understood. Microbial laboratory experiments observed a shift of 1-2‰ in $^{238}U/^{235}U$ during reduction experiments with multiple bacterial strains (Basu et al., 2014). Little work has been done to determine the isotopic fractionation induced by abiotic reduction.

This study focuses on determining the isotopic fractionation of uranium during reduction with Fe(II). Time series experiments with aqueous Fe(II) and magnetite (Fe_3O_4) were conducted with samples analyzed by a MC-ICP-MS. We find aqueous Fe(II) has a limited ability to reduce U(VI) and no isotopic data is reported. Stoichiometric magnetite ($\chi=0.49$) reduces U(VI) to U(VI) completely and rapidly as confirmed by XANES analysis, but induces no shift in $^{238}U/^{235}U$. This finding matches

results observed in three other studies (Rademacher et al, 2006; Stirling et al., 2007;

Stylo et al., 2014) where abiotic reduction does not induce isotopic fractionation.

Acknowledgments

I owe the success of this project to many individuals. I especially thank my advisor Dr. Tom Johnson for providing me the opportunity to pursue research in the field of isotope geochemistry and guide me through the many challenges faced while conducting these experiments. I would also like to thank Dr. Craig Lundstrom and Dr. Alyssa Shiel for their endless help and support while working in the lab and on the mass spectrometer. My gratitude goes to Dr. Maxim Boyanov from Argonne National Lab for the opportunity to conduct and analyze my experiments at the Advanced Photon Source. Additionally, I would like to thank the rest of our geochemistry group Gideon Bartov, Anirban Basu, Norbert Gajos, Nick Huggett, Noah Jemison, Kelsey Kehoe, Xiangli Wang, and Katelyn Zatwarnicki for their help and support, and making the lab a fun and enjoyable environment. Thank you to the Department of Geology for their continued support, specifically, Shelley Campbell, Lana Holben, and Marilyn Whalen for their gracious help beyond the laboratory. And finally, thank you to my family, Ed and Ruby Grimm, and F. Alexa Warburton, and friends for constant support while pursuing my degree.

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Introduction

Uranium (U) is found at low concentrations in nearly all rocks, soils, and water. U is mined and refined for military applications, including nuclear weapons and armor, and nuclear energy production. In the past twenty-five years, the environmental geochemistry of U has gained increasing attention due to its toxicity and mobility in groundwater. Although it is radioactive, its decay is extremely slow and toxicity unrelated to radioactivity is the major health concern (Domingo, 2001). Under the EPA's Safe Drinking Water Act, the maximum contaminant level of uranium is 30 µg/L (U.S. EPA). The major source of contamination is through chemical leaching from mill tailings (Abdelouas, 2006). Today there are approximately eighty U-related facilities being decommissioned within the United States, including thirty-eight active or former U mill tailings sites (U.S. NRC).

U exists in two stable oxidation states, hexavalent U (U(VI)) and tetravalent U (U(IV)), in natural aqueous systems. Under oxidizing conditions, U(VI) is soluble and mobile as the uranyl ion (UO_2^{2+}) whereas under reducing conditions and circum-neutral pH, U(IV) is much less soluble, essentially immobile, and not bioavailable (Langmuir, 1978). Uranium can change from one oxidation state to another by the addition or removal of electrons; these redox transformations are important as they govern the bioavailability of uranium in the natural environment. Of particular importance to groundwater contamination management is that fact that U can be reduced from mobile U(VI) to immobile U(IV) by microbial and abiotic chemical reactions (Ginder-Vogel et al., 2008). A diverse group of bacteria are known to successfully reduce uranium through coupling with an electron donor such as acetate, including various strains of iron and

sulfate reducing bacteria (Wall, 2006). Fe(II) species, such as aqueous Fe(II), magnetite, green rust, siderite, or iron(II)-doped hematite are known to reduce U(VI) (Du et al., 2011; Ithurbide et al, 2009; Jang et al., 2008; Latta et at., 2012; O'Loughlin et al, 2003). Also, sulfide minerals such as galena or pyrite are known to reduce U(VI) (Wersin et al., 1994).

During bioremediation, microbial reduction by existing communities is stimulated by providing an electron donor (Anderson et al., 2003; Williams et al., 2011). The oxidation of the electron donor can be coupled to the reduction of uranium via microbial metabolisms. However, aside from such direct microbial reduction of U(VI), reduction of Fe(III) and SO_4^{2-} also occurs, and the reaction products, Fe(II) and S(-II), can also reduce U(VI). Bioremediation has been successfully achieved at the U.S. Department of Energy's biostimulation projects in Rifle, CO and Oak Ridge, TN where uranium was removed from groundwater by a series of stimulated bioremediation experiments (Anderson et al., 2003; Williams et al., 2010; Wu et al., 2006). It is not clear if the U(VI) reduction occurs via direct microbial action or via abiotic reduction resulting from microbial production of Fe(II) and/or S(-II).

The extent of U(VI) reduction is typically assessed via concentration measurements, but this approach can be compromised by the many processes that influence concentration. U removal from groundwater can occur through different mechanisms such as reduction of U(VI) during biostimulation or adsorption of U(VI). Both cause decreases in concentration such that using concentration measurements does not distinguish between the two mechanisms of removal. Similarly, minor changes in water chemistry can induce desorption, which can mask decreases due to reduction.

However, U isotope ratio ($^{238}U/^{235}U$) measurements can be used to detect and quantify U(VI) reduction due to the fractionation of isotopes (Basu et al, 2014, Bopp et al, 2010; Brennecka et al., 2010; Stirling et al., 2007; Weyer et al., 2008). Uranium's two most abundant isotopes, ^{238}U ($t_{1/2}$ = 4.47 x 10^9 years) and ^{235}U ($t_{1/2}$ = 0.7 x 10^9 years), have natural abundances of 99.27% and 0.72% respectively. In groundwater studies with time scales of months or years, these two isotopes can be treated like stable isotopes, as their half-lives are many orders of magnitude longer. Variations in the $^{238}U/^{235}U$ ratio are now measured by high precision mass spectrometry. Because the variations are small, they are expressed as per mil deviations from an inter-laboratory standard (CRM-112A, New Brunswick Laboratory) and reported in delta notation as defined by

$$\delta^{238}U = (\frac{R_{Sample}}{R_{CRM-112A}} - 1) * 1000‰ \tag{1}$$

where R is the measured ratio of $^{238}U/^{235}U$.

The $^{238}U/^{235}U$ ratio may be altered by chemical processes because of slight differences in the chemical properties between the two isotopes. Theoretical predictions by Bigeleisen (1996) and Schauble (2007) state that if isotopic equilibrium is attained, ^{238}U will preferentially partition into the species with the lower oxidation state, U(IV), and the coexisting U(VI) will be enriched in ^{235}U. This enrichment is opposite to the fractionation observed with other common redox-active elements (e.g. C, N, S, Cr, Fe, Se), where the heavy isotopes are enriched in the phase with the higher valence because of differences in vibrational frequencies and energies of bonds as a function of mass. U isotope ratios are also affected by this mass-dependent fractionation, but a second, stronger effect dominates. This second phenomenon is known as the nuclear

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volume effect (Bigeleisen, 1996; Schauble, 2007), which is significant only in very heavy elements with large nuclei.   The size of the nucleus is so large that certain electron orbitals are affected and the orbital energies vary with nuclear size. Because electronic configurations of U(VI) and U(IV) differ, and because orbital energies depend on nuclear volume, ^{238}U, with its larger nucleus, and ^{235}U, with its smaller nucleus, partition unequally between the two valences at equilibrium. The lowest energy state is attained when the larger isotope, ^{238}U, is enriched in species with the lower electron density at the nucleus, which is U(IV). This nuclear volume effect opposes mass-dependent U isotope fractionation, and is considerably larger. Accordingly, the net effect is an isotopic fractionation opposite in direction to the classic mass-dependent fractionation observed with light elements. Equilibrium U isotope fractionation between dissolved U(VI) and U(IV) has been measured in laboratory experiments; the U(IV) is enriched in ^{238}U by 1.64 ‰ (Wang, 2013).

Similar theory that explores U isotope fractionation induced by kinetically limited redox reactions has not been developed. However, isotopic fractionation should also be governed by the same general phenomenon, and it seems likely that ^{238}U(VI) should be reduced at a slightly greater rate relative to ^{235}U because of the chemical energy differences involved as valence electrons are added. The magnitude of fractionation is given by the parameter α, which is defined

$$\alpha = \frac{R_{Product\ Flux}}{R_{Reactant}} \tag{2}$$

where $R_{Product\ Flux}$ and $R_{Reactant}$ are the ^{238}U/^{235}U ratio in the U(IV) product flux (the incremental product at one instant in time) and remaining U(VI) pool, respectively. α

can be conveniently expressed as a per mil quantity using the parameter ε, which is defined

$$\varepsilon = 1000\text{‰}*(\alpha - 1) \tag{3}$$

Kinetic U isotope fractionation has been observed in laboratory experiments with four different bacteria; the product U(IV) was found to be enriched in ^{238}U relative to the reactant U(VI) (Basu et al., 2014). U(VI) was reduced to U(IV) by *Geobacter Sulfurreducens* strains PCA and Criddle; measured ε values were 0.68‰ and 0.95‰ respectively. *Anaeromyxobacter dehalogenans* strains FRCW and FRC-R5 yielded ε values of 0.75‰ and 0.98‰. *Desulfitobacterium* Viet1 yielded an ε value of 0.84‰ and *Shewanella sp.* (Neckar River) yielded an ε value of 0.96‰.

Occurrence of similar U isotope fractionation in natural settings is supported by other studies where the product U(IV) was found to be enriched in ^{238}U (Bopp et al, 2010; Brennecka et al., 2010; Weyer et al., 2008). At the U.S. Department of Energy's biostimulation project in Rifle, Colorado, a decrease of roughly 1‰ in the ^{238}U/^{235}U ratio of the dissolved U(VI) was observed during the decrease in U(VI) concentration in response to acetate injections (Bopp et al., 2010). In natural rock samples, isotopic fractionation has also been observed in roll front deposits (Bopp et al., 2009; Brennecka et al., 2010) and black shales (Weyer et al., 2008).

These findings support the theory that during U(VI) reduction, isotopic fractionation and enrichment of ^{238}U in the product U(IV) does occur. They also allow us to monitor the extent of reduction, as the remaining U(VI) in solution will become progressively enriched in ^{235}U as reduction proceeds. Thus, we can use the change in the ^{238}U/^{235}U ratio to determine the extent of reduction. Because concentration changes

can be unreliable indicators of reduction, having a robust way to determine the extent of reduction is necessary, especially in a bioremediation setting. The Bopp et al. (2010) study demonstrates we can monitor reduction over time. However, in order to quantify reduction in all relevant geochemical systems, the magnitude of fractionation must be understood for all potential reduction mechanisms. The Basu et al. (2014) study found a diverse community of microbial bacteria can cause fractionation of U isotopes during reduction. However, three studies of abiotic U(VI) reduction, found a lack of fractionation of U isotopes during reduction (Rademacher et al., 2006; Stirling et al., 2007; Stylo et al., 2014). Therefore, it is clear fractionation of U isotopes is not well understood and more studies are needed to understand fractionation for all potential U(VI) reduction mechanisms.

The goal of this study was to determine the shift in $^{238}U/^{235}U$ induced by abiotic reduction by aqueous Fe(II) and magnetite (Fe_3O_4). Aqueous Fe(II) could have a significant role in the reduction of uranium during bioremediation. When Fe(III) oxyhydroxide phases are reduced during microbial stimulation, a flux of aqueous Fe(II) is introduced into the system. One published study reports reduction of U(VI) by Fe(II) (Du et al., 2011). Magnetite could also have a significant role in U(VI) reduction. This mineral is common in many settings and has been shown to reduce U(VI), though partial oxidation renders it non-reactive (Latta et al., 2012). It can also be recharged, increasing the amount of available Fe(II), by the adsorption and incorporation of aqueous Fe(II) into its structure. To determine U isotope fractionation during reduction, we conducted experiments where sampling occurred at various time points. Individual

samples were analyzed for U isotopes and change in U(VI) concentration to determine

the overall shift in $^{238}U/^{235}U$ over the duration of the reaction.

Materials and Methods

Both experiments required the use of similar techniques for reactor construction and sampling. Exclusion of O_2 was crucial; it was removed by degassing all required solutions with ultrapure N_2 that was passed through a copper O_2 scrubber. Reactions were conducted in either 120 mL glass serum bottles (aqueous Fe(II) experiments) or 25 mL glass test tubes (magnetite experiments) and sealed with rubber septa (CLS Butyl Rubber, 20mm). All stock solutions were prepared with ultrapure (18 MΩ-cm, Millipore) deionized water. For aqueous Fe(II) experiments, prior to sampling, syringes were purged by N_2, followed by injection of N_2 into the reactions to maintain positive pressure during sampling. Magnetite experiments were conducted in a 95% N_2, 5% H_2 anaerobic glovebox (Coy Laboratories Products). A palladium catalyst was used to remove O_2, with low concentrations confirmed by resazurin color indicator solution kept in the glove box at all times.

Chemical Synthesis. An Fe(II) stock solution was synthesized following the method of Rakshit el al. (2008). 0.56 g of iron metal was dissolved in 100 mL 0.2 M HCl in an anaerobic serum bottle. The bottle was lightly heated, with a needle piercing the rubber septum to release the evolved H_2 gas. Once the Fe^0 was consumed (~ 2.5 hours), a clear (no color and solids) solution of $FeCl_2$ was obtained. Afterwards, the concentration of Fe(II) was determined the by the ferrozine colorimetric method (see below).

Stoichiometric magnetite (Fe_3O_4, $\chi = 0.49$) was synthesized by following published methods (Gorski and Scherer, 2009; Latta et al., 2012; Latta et al., 2014;

Regazzoni et al., 1981). A mixture of 0.1 M Fe(II) and 0.2 M Fe(III) was prepared under anoxic conditions in the glovebox. NaOH was titrated into the solution drop-wise to increase the pH to greater than 10, and the solution was stirred overnight as magnetite precipitated. Afterwards, the solution was filtered and dried in the glovebox. The crystals were characterized by the ferrozine colorimetric method (see below) to determine the Fe(II):Fe(III) ratio.

A U(VI) stock solution was prepared by dissolving U metal (isotopic standard CRM-112a) in ultrapure concentrated HNO_3. The resulting uranyl nitrate was dried down completely, re-dissolved, and dried down in concentrated HCl. The resulting uranyl chloride was dried down completely and re-dissolved in a degassed 100 mM $NaHCO_3$ solution to prepare a ~4400 mg/L uranyl carbonate stock. A 340 mg/L uranyl carbonate solution was prepared by the dilution of the uranyl carbonate stock with ultrapure deionized water and used for all reactions.

Experimental Design. U(VI) reduction experiments with aqueous Fe(II) had reaction conditions that were chosen to roughly simulate natural groundwater; the pH was buffered at 6.5 by 8 mM $NaHCO_3$. Fe(II) stock solution was added to the degassed buffer solution to attain a concentration of 0.840 mM and allowed to equilibrate for 24 hours. The reaction was initiated by the addition of U(VI) stock solution to attain a U concentration of 0.042 mM.

Sampling occurred at 7 time points over 2.5 months. During sampling, aliquots of aqueous phase U(VI) were removed, and immediately acidified to a pH < 5.5. This stopped the reduction reaction, which is not thermodynamically favorable below pH 5.5

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(Du et al., 2011). Samples were stored for up to 7 days before being prepared for concentration and isotopic analysis.

Reduction experiments with magnetite had reaction conditions that were similar to natural groundwater in which the pH was buffered at 7.2 by 2 mM of $NaHCO_3$. Magnetite suspensions (~2.75 g/L) were prepared by re-suspending carefully weighed solids (53.9 mg ± 0.9 mg (experiment 1) and 60.1 mg ± 1.1 mg (experiment 2)) in 18.5 mL of buffer solution. The buffer solution was allowed to equilibrate with the magnetite for 24 hours. Following equilibration, U(VI) stock solution was added to attain a concentration of 0.05 mM. In all reactions, the amount of Fe(II) in the magnetite was much greater than that needed to reduce all of the U(VI). Time series experiments were conducted, in which several identical reaction tubes were created and injected with U(VI) at the same time. Reaction tubes were opened and processed at various time points to observe reduction over 24 hours. At each time point, the contents of a reaction tube was filtered (0.2 µm), the filtrate was collected, and the filters holding the solids were exposed to a 0.5M $NaHCO_3$ bath and shaken for 24 hours to ensure complete desorption of U(VI). After 24 hours, the bath was filtered (0.2 µm), and the solution was collected and stored for isotopic analysis. Four additional experiments, with increased reaction volumes (100 mL) that contained the same magnetite g/L suspension and Fe(II):U ratio as experiments 1 and 2, were conducted and analyzed via X-ray spectroscopy to confirm that U(VI) concentration decreases were caused by reduction and to confirm complete U(VI) recovery by the $NaHCO_3$ bath.

Analytical Methods: Fe(II) Concentration. The ferrozine method was used to determine the concentration of the Fe(II) stock and measure the Fe(II):Fe(III) of magnetite via a visible light colorimetric method (Viollier et al, 2000). Fe(II) in solution complexes strongly with ferrozine and the complex has a strong purple color at pH between 4 and 9.5. A Thermo Genesis Spectrophotometer at a visible light wavelength of 562 nm was used to measure the concentration of the complex. The method has a working range of 0.1 µM to 50 µM (± 0.09 µM, 2σ).

To determine the stoichiometry of the magnetite, solids were dissolved in 4.5N HCl inside the glove box to obtain a total Fe concentration of 10mM. Fe(II) and total Fe were measured to allow determination of the Fe(II):Fe(III) ratio of the magnetite. Fe(II) concentration was measured via direct measurement of the solution after addition of ferrozine, whereas total Fe was measured after a hydroxylamine step, reducing Fe(III) to Fe(II). This was achieved by adding an aliquot of 1.4M hydroxylamine in 2M HCl to the sample. All solutions were processed within two minutes after removal from the glove box to ensure no oxidation of Fe(II).

Analytical Methods: Uranium Concentration. A Nu Plasma HR MC-ICP-MS running in low resolution mode was used to measure preliminary uranium concentrations needed to determine the amount of uranium double spike needed for high precision isotope analysis. Concentrations were determined by comparing the measured [238]U beam intensity of a 10 µg/L standard to that of diluted experimental samples. Final concentrations reported were determined by isotope dilution calculations from isotope measurements (see below).

Analytical Methods: U Isotope Ratio Measurements. A ^{233}U-^{236}U double spike method (Basu et al., 2014; Bopp et al., 2010; Shiel et al., 2012; Stirling et al., 2007; Weyer et al., 2008) was used to determine the $^{238}U/^{235}U$ for dissolved U(VI), adsorbed U(VI), and magnetite free controls. The double spike ($^{233}U/^{236}U$ ratio of ~ 0.46) was prepared in house with ^{233}U and ^{236}U spikes. The spike provides a way to correct instrumental mass bias and/or possible isotopic fractionation during sample preparation. A precise amount of spike was added to each sample to obtain a $^{238}U/^{236}U$ of ~28. Each spiked sample was evaporated to dryness, re-dissolved in 3.0 N HNO_3, and purified via UTEVA anion exchange resin (Eichrom) (Weyer et al., 2008). Briefly, 0.2 mL UTEVA resin was cleaned, and conditioned with 5 mL of 0.05 M HCl and 1 mL of 3 N HNO_3 respectively. Samples were then passed through the columns; U(VI) was retained. Matrix elements were eluted with 4 mL of 3 N HNO_3 followed by release of U from the resin with 2.4 mL of 0.05 N HCl. Finally, the purified U samples were evaporated to dryness, then re-dissolved in 2% HNO_3 and analyzed on the MC-ICP-MS. Samples were compared to a known isotope standard (CRM-112A, New Brunswick Laboratory) and reported in delta notation (eqn 1). The CRM-112a standard was measured every three samples to monitor drift of the standard due to changing deviation of the mass bias from an ideal exponential law. Two other isotope standards, REIMEP 18-A (IRMM) and CRM-129A (New Brunswick Laboratory) were also measured during each run. A more detailed description of sample routine and instrumental parameters can be found in Shiel et al. (2013).

Precise U concentrations were determined by an isotope dilution calculation; the calculation is based on the measured $^{238}U/^{236}U$ of the sample and compared to the

amount of carefully weighed spike and sample, and known spike U ratio to determine

the concentration of U. External precision (95% confidence) for δ^{238}U were ± 0.05 ‰

and ± 0.04 ‰ for experiment 1 and 2 respectively determined from 9 pairs of samples

prepared in duplicate, using a modified root mean square calculation:

$$2\sigma = 2 \cdot \sqrt{\frac{\sum_{i=1}^{n}(i_a - i_b)^2}{2 \cdot n}} \tag{4}$$

where a and b are two duplicate analyses of sample i, and n is the number of duplicated

samples (n = 9). The external uncertainties for U concentrations (eqn 4) were 1.2% (n =

9) and 0.5% (n = 9) for experiment 1 and 2 respectively. The mean δ^{238}U$_{112a}$ values of

REIMEP 18-A and CRM-129A were -0.17 ‰ ± 0.07 ‰ and -1.74 ‰ ± 0.07 ‰

respectively.

Analytical Methods: X-ray Absorption Spectroscopy of Magnetite Experiments.

Four experiments with magnetite were constructed with conditions that were the same

as experiments 1 and 2 except for more U and magnetite. Magnetite suspensions

(~2.70 g/L) were prepared by re-suspending carefully weighed solids (266.4 mg ± 2.0

mg) in 96.25 mL of buffer solution. The amount of magnetite and reaction volume was

increased to obtain a desired U concentration of 3000-4000 ppm and 300 mg of

magnetite required to obtain sufficient X-ray absorption. The buffer solution was

allowed to equilibrate with the magnetite for a minimum of 24 hours. Following

equilibration, U(VI) stock solution was added to attain a concentration of 0.05 mM. At

four time points (1, 3, 8, and 24 hours), the experiments were stopped by filtration

through 0.2 micron nylon membrane filters. The solids were packed into a 1.5 mm thick

drilled plexiglass sample holder with Kapton film windows. All sample manipulations

were done inside an anoxic glove box (4% H_2/balance N_2, Pd/C catalyst) and sample transport and measurement was done in sealed anoxic containers. Analysis of samples was not immediate where reactors 1, 2, 3, and 4 were not measured until 1.25, 2.5, 6.5, and 6 hours after filtration respectively.

The following x-ray procedure was provided by Maxim Boyanov, Argonne National Lab. Uranium L_{III}-edge (17,166 eV) x-ray absorption near-edge (XANES) was collected at room temperature in fluorescence mode at sector 10-ID, MRCAT/ EnviroCAT beamline (Segre et al., 2000), Advanced Photon Source, Argonne National Laboratory, Illinois, USA. The incident energy was scanned by using the Si(111) reflection of the double-crystal monochromator in quick-scanning mode (~ 30 seconds). Energy calibration was maintained at all times by simultaneously collecting a spectrum from a hydrogen uranyl phosphate standard using x-rays transmitted through the samples.

Normalization and background subtraction of the measured spectra was done with the program AUTOBK (Newville et al.,1993). Data were Fourier transformed (FT) by using the FEFFIT program (Newville et al., 1995). Identical transform parameters were used for the standards and the unknown spectra (e.g., k-weighting, Fourier transformation and fitting ranges, and 1.0 $Å^{-1}$ Hanning window functions).

Results

U(VI) Reduction by Aqueous Fe(II). For the experiment with aqueous Fe(II), dissolved U(VI) concentration decreased very slowly, and incomplete removal of U(VI) from solution was observed (Fig. 1). After 68 days, the reaction had attained reduction of ~25% of the initial U(VI). Given the analytical uncertainty of 10%, it is not possible to be sure the reaction had stopped, but the overall trend suggests a slowing of U(VI) removal. Several preliminary experiments (data not shown) with combinations of lower $NaHCO_3$ concentration (4 mM), lower aqueous Fe(II) concentrations, or addition of Mg (0.5 mM) produced the similar observation that reduction did not advance beyond ~25%.

Isotopic data were not collected for this experiment due to the limited amount of U(VI) reduction. In order to collect precise U isotope data, significant reduction is necessary. When reduction is limited, analytical uncertainty is greater. Therefore, it would be difficult to distinguish if a shift in U isotopes were due to isotope fractionation or analytical uncertainty.

U(VI) Reduction by Magnetite: Concentration and XANES results. Complete removal of U(VI) from solution and subsequent reduction to U(IV) was observed for both isotopic analysis experiments involving magnetite reduction (Fig. 2). Both magnetite-free control samples for experiments 1 and 2 showed no decrease in U(VI) concentration over time. These two reactors thus confirmed that change in U(VI) concentration were due to reduction by magnetite and not due to sorption to the glass test tubes.

Determining the speciation of U in these experiments was not straightforward. During U(VI) reduction by magnetite, adsorption of dissolved U(VI) is required for U(VI) reduction. In order to determine the extent of reduction, desorption of the adsorbed U(VI) is necessary, and was achieved during sample processing by the bicarbonate extraction. Following the extraction, magnetite free controls, dissolved U(VI), $NaHCO_3$-extracted U(VI) (adsorbed U(VI)) were used to determine the amount of U(VI) reduction for each individual experiment. The magnetite free controls for each experiment were used to determine an average initial U(VI) concentration where samples were removed from each control to monitor the concentration at each time point. Experiment 1 and 2 had initial U(VI) concentrations of 12.59 mg/L ± 0.26 mg/L and 12.48 mg/L ± 0.09 mg/L respectively. The amount of U lost from solution was calculated by subtracting dissolved U(VI) from the initial U(VI). This was followed by determination of the concentration of U(IV), by subtraction of the $NaHCO_3$-extracted U(VI) from the bath from the total U(VI) lost from solution. Thus, the U(IV) concentration is obtained by the formula

$$[U(IV)] = [U(VI)_{Initial}] - [U(VI)_{Dissolved}] - [U(VI)_{Extracted}] \qquad (5)$$

The decrease in U(VI) concentration roughly followed a first-order kinetic model where U(VI) half-lives were 2.5 hours (experiment 1) and 0.5 hours (experiment 2). Overall, the uptake and reduction rate was much slower for experiment 1(Fig. 2). This was expected, as surface area was significantly increased for experiment 2 (see discussion). For experiment 1, within 1 minute, 25% of U(VI) in solution was lost (sample 1) from solution. Uptake of U(VI) continued quite rapidly where 83% was removed from solution in 6 hours. Afterwards, uptake was considerably slower as the

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remaining 17% was removed over 18 hours. Throughout uptake, the amount of adsorbed U(VI) did not get above 25% of total U in the reactor (Table 1). In the first hour, the amount of adsorbed U(VI) increased until a peak at 1 hour (2.93 mg/L ± 0.26 mg/L), followed by a steady decrease as more adsorbed U(VI) was reduced to U(IV) and uptake of U(VI) from solution decreased. Reduction was quite rapid, with a faster rate than the uptake and adsorption of U(VI), as the amount of U(IV) was always greater than the adsorbed pool of U(VI) (Table 1). In the beginning of the reaction, within 1 minute, 17% of the adsorbed U(VI) was reduced to U(IV). Possibly, the bicarbonate extraction did not recover all of the adsorbed U(VI), resulting in more in the assumed reduction pool. Reduction continued quite rapidly, reaching 76% U(VI) reduced after 6 hours, then slowed considerably where the remaining adsorbed U(VI) was reduced to U(IV) over 18 hours.

For experiment 2, within 1 minute, 35% of dissolved U(VI) was removed from solution (sample 1) due to adsorption (Table 1). Uptake of dissolved U(VI) by adsorption continued quite rapidly; 90% was removed from solution in 3 hours. Afterwards, uptake was considerably slower, as the remaining 10% was removed over 21 hours. Throughout uptake, the amount of adsorbed U(VI) did not get above 27% of total U in the reactor. In the first hour, the amount of adsorbed U(VI) increased until a peak at 1 hour (3.40 mg/L ± 0.09 mg/L), followed by a steady decrease as more adsorbed U(VI) was reduced to U(IV) and uptake of U(VI) from solution decreased. Reduction was quite rapid, as the amount of U(IV) was always greater than the adsorbed pool of U(VI) (Table 1). In the beginning of the reaction, within 1 minute, 15% of the adsorbed U(VI) was reduced to U(IV). Reduction continued quite rapidly,

reaching 75% U(VI) reduced after 3 hours then slowed considerably where the remaining adsorbed U(VI) was reduced to U(IV) over 21 hours.

U XANES data (Fig. 3) indicate our 4 X-ray analysis reactors contain U(IV) associated with magnetite. Comparing the reactors to U(VI) and U(IV) standards, the edge position and shape is similar to the U(IV) standards. A qualitative comparison between the U(IV) standards and reactor spectra determined that U is approximately 90-95% $U(IV)/U_{Total}$. However, there is a spectral difference, where there is a greater absorbance in the post-edge region relative to the standards, indicated by an arrow on the figure. It is possible the broadening could be explained as adsorbed U(VI); however, we believe this to not be the case. The U(VI) characteristic resonance feature resulting from the nearest neighbor oxygens in the uranyl group (Hudson et al., 1995) is not observed in the measured reaction samples. Also, there is no change in slope of the reactors samples compared to the successive change in the U(VI) standard slope. We believe the greater absorbance, and broadening, is due to compression/disorder of the O shell around U(IV) relative to the observed O shell in uraninite.

U(VI) Reduction by Magnetite: Isotopic results. During the isotopic analysis experiments, there were slight variations in $\delta^{238}U$ over time (Fig. 2). Both magnetite free control samples, for experiments 1 and 2, did not change in $\delta^{238}U$ over time (Fig. 2). These two reactors confirmed that $\delta^{238}U$ changes in magnetite bearing experiments were either due to adsorption onto or reduction by magnetite. In experiment 1, we observed no significant change in $\delta^{238}U$ over time for the adsorbed U(VI) samples (Fig. 2, 4). However, the first sample delta value was slightly enriched in [238]U and

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subsequent samples continued to average around 0.15 ‰ ± 0.05 ‰ as reduction continued. $\delta^{238}U$ for dissolved U(VI) did not change over time and remained around 0.00 ‰. Sample 7 and 8 (6 and 24 hours respectively) were not included due to increased error observed in $\delta^{238}U$ during the instrumental analysis.

In experiment 2, we also observed no significant change in $\delta^{238}U$ over time for the adsorbed U(VI) (Fig. 2, 4). However, the first sample delta value was slightly enriched in ^{235}U and subsequent samples continued to average around - 0.10 ‰ ± 0.04 ‰ as reduction proceeded. $\delta^{238}U$ for dissolved U(VI) fluctuated with time. In the beginning of the experiment, $\delta^{238}U$ was lighter (- 0.12 ‰), but as the reaction progressed and essentially all of dissolved U(VI) was removed, $\delta^{238}U$ increased to a $\delta^{238}U$ of + 0.12 ‰. Interestingly, samples 6 and 7 (6 and 12 hours respectively) contained 0.3% and 0.1% of the initial dissolved U(VI), yet, the extent of reduction was not significantly different from previous samples. It is possible that the adsorbed U(VI) pool has significantly affected the extremely small remaining concentration of dissolved U(VI) due to the decreased rate of adsorption and reduction. Sample 8 was excluded due to increased error in $\delta^{238}U$ during instrumental analysis caused by insufficient signal from the extremely low concentration of U. Although we observed minor variations in $\delta^{238}U$ for experiments 1 and 2, we did not observe any major isotopic fractionation during reduction.

Discussion

U(VI) Reduction by Aqueous Fe(II). In this experiment, results were similar to those of most of the previously reported studies of U(VI) reduction by Fe(II), in which reduction with aqueous Fe(II) either did not occur or ceased prior to completion (Du et al., 2011; Fredrickson et al., 2000). The sole exception was one experiment by Du et al. (2011) who reported they were able to successfully reduce U(VI) completely with Fe(II), in the form of siderite, at high pH conditions. It should be noted that reaction conditions and parameters that may have influenced the success of the reaction in previous studies that varied considerably and differed from those of the present study. Du et al. (2011) noted that thermodynamics plays a significant role in this reaction; ΔG and forward progress of the reaction are significantly affected by the concentrations of $NaHCO_3$, U, and Fe(II). They designed their experiments to enhance the thermodynamic potential for reaction. In the present study, reaction conditions were chosen to reflect typical groundwater conditions, rather than conditions that are non-natural but thermodynamically favorable.

When comparing the Du et al. (2011) and Fredrickson et al. (2000) experiments with each other and with those of the present study, it appears $NaHCO_3$ concentration plays a major role in controlling the amount of reduction. It is known that bicarbonate complexes strongly with U (Langmuir, 1978). This reduces the activity of U(VI) and therefore decreases the thermodynamic drive. In the Fredrickson et al. (2000) study, no detectable reduction was measured when the reaction was buffered with 30 mM $NaHCO_3$ and contained 2 mM aqueous Fe(II) and 1 mM uranyl acetate. However, under conditions using the PIPES buffer, rather than $NaHCO_3$, approximately 20%

reduction was observed. In the Du et al. (2011) study, a reaction at pH 6.38 induced approximately 37% reduction when the reaction contained 5 mM $NaHCO_3$, 1 mM aqueous Fe(II), and 0.2 mM U(VI). Like this study, they also observed a plateau in their reaction; reduction ceased when 37% of the U(VI) was consumed. Complete reduction was observed at pH 10.73 with the same proportions of reagents. These findings demonstrate $NaHCO_3$ concentration significantly affects the amount of reduction. However, the Fe(II) concentration and speciation also appear to affect the amount of reduction. The Du et al. (2011) study predicts the relative abundances and speciation of U and Fe for the reaction conditions. At low pH, aqueous Fe(II) is the dominant species but siderite ($FeCO_3$) becomes the dominant species above pH 6.5. Taking this into account, it is clear that siderite must be the reductant that completely reduced U(VI) at high pH. However they state that siderite cannot reduce U(VI) below a pH of 9.5. Therefore, this suggests that in their pH 6.38 experiment, the amount of U(VI) reduced was limited by the amount of available aqueous Fe(II) (~15-20% of the initial aqueous Fe(II) due to precipitation of siderite), and not by the concentration of $NaHCO_3$.

In the present study, Fe(II) speciation was taken into account and the experiments were adjusted to contain higher concentrations of aqueous Fe(II) to completely reduce U(VI). In preliminary experiments (data not shown) lower concentrations of aqueous Fe(II) resulted in very little reduction. Following those findings, the concentration of aqueous Fe(II) was increased to create reaction conditions where the potential for complete reduction was possible. However, regardless of $NaHCO_3$ or aqueous Fe(II) concentrations, complete reduction was still not possible. As Du et al. (2011) suggested, thermodynamics play a significant role,

and the reaction is not thermodynamically favorable with 5mM NaHCO$_3$ present except under a narrow range of conditions. First, at pH greater than 6.5, siderite forms and drives the Fe(II) concentration down to such low concentrations that the reaction is very slow. However, U(VI) reduction by siderite is not thermodynamically favorable below pH < 9.2, and thus is not of interest in most groundwater systems. As Du et al. (2011) calculated, below a pH of 5.5, the reaction was thermodynamically not favorable in their experiments. Therefore, reduction by aqueous Fe(II) was only possible in a narrow range of pH, high enough so that the thermodynamic drive is favorable, but not so high so that siderite precipitation drives Fe(II) concentration so low that the thermodynamic drive and/or kinetics are not favorable. The formation of siderite depends on the concentrations of Fe(II) and NaHCO$_3$ and occurs at pH > 6.8 for our experiments. Even within the favorable pH range, lower concentration of either Fe(II) or U(VI) makes the reaction less favorable. In the present study, it appears that as the reaction progressed, the thermodynamic drive evolved to $\Delta G=0$, and the reaction stopped at around 25% reduction of the initial U(VI).

Overall, the results of the present study reinforce and extend the conclusions of Du et al. (2011), who demonstrated that U(VI) reduction by aqueous Fe(II) is limited depending on the reaction conditions. Our experiments were designed to be closer to conditions expected at relevant natural sites, whereas the Du et al. (2011) experiments had much greater U and Fe concentrations. Our results suggest that, under typical groundwater conditions, insufficient reduction occurs to drive U(VI) concentrations below regulatory levels, and aqueous Fe(II) is not a viable candidate as a mechanism of U(VI) reduction. On the other hand, Fe(II) can nonetheless be an important reductant in

other forms, as it can be incorporated or adsorbed onto the surfaces of Fe-bearing minerals (Fredrickson et al., 2000; Larese-Casanova and Scherer, 2007; Latta et al., 2012). Attention should be focused on these minerals, rather than aqueous Fe(II), and thus the focus of the present study was mostly on U(VI) reduction by magnetite.

U(VI) Reduction by Magnetite: Extent of Reduction. In both of the experiments that yielded the isotopic analyses, the rate of U reduction was similar to that observed in several previous studies - complete reduction of U(VI) to U(IV) occurred within 48 hours (Latta et al., 2012; Latta et al., 2014, Singer et al., 2012a; Singer et al., 2012b; Veeramani et al., 2011). Other studies have reported slower rates or incomplete reduction, but those experiments had conditions where the Fe:U ratio was much smaller or not well constrained, or the Fe(II)/Fe(III) ratio of magnetite was less than 0.38 where little to no reduction occurs (Missana et al., 2003; O'Loughlin et al., 2010; Regenspurg et al., 2009; Scott et al., 2005; Veeramani et al., 2011).

The two duplicate experiments in this study differed significantly in the rate of reduction (Fig. 2). The reduction rate in experiment 1 was approximately half that of experiment 2 and is attributed to the larger clumps of magnetite used. No washing and minimal processing of magnetite was done after its synthesis, to limit the amount of Fe(II) loss. Prior to experiment 2, the magnetite was crushed, in an O_2-free environment, to a finer powder. This accounts for the faster reaction rate due to more surface area for the same amount of magnetite.

The XANES results indicate greater extents of reduction for earlier time points, relative to the extents of reduction derived from experiments 1 and 2.   However, most

or all of this difference probably results from the fact that the XANES measurements were subject to a time lag between sampling of the experimental vessels and the actual x-ray measurements. In the isotopic analysis experiments, U(IV) increased over time as the reaction progressed. The earlier time points show 34-53% and 57-74% reduction of U(VI) at 1 and 3 hours, respectively. In the XANES analysis experiments, all 4 samples (1, 3, 8, and 24 hours) contained approximately 90-95% U(IV). For the isotopic analysis concentrations, each reaction is stopped by filtration followed by immediate exposure to the 0.5 M NaHCO$_3$ bath. For the XANES analysis, the solution and magnetite were separated by filtration. However, further processing/analysis after filtration does not occur for 1.25, 2.5, 6.5, and 6 hours for reactors 1, 2, 3, and 4 respectively. We believe that this time lag for the samples prepared for XANES analysis allowed the remaining adsorbed U(VI) to be reduced to U(IV) after filtration and prior to analysis. Therefore, we conclude that the additional reduction is creating the difference in the observed U(IV) concentrations as the XANES analysis does not record the speciation of U at the time of filtration. Although the XANES analysis cannot accurately track the U(VI):U(IV) ratio during the experiments, it clearly confirms the magnetite used in this study rapidly reduced U(VI) and the loss of U(VI) from solution was not just adsorption.

U(VI) Reduction by Magnetite: Bicarbonate Extractions. Using bicarbonate to extract adsorbed U(VI) could affect the calculation of the extent of reduction. It is possible the high concentration of bicarbonate altered the system, and the extraction either recovered U(IV) or, on the other hand, underestimated the amount of adsorbed U(VI). Recently, bicarbonate extraction has been linked to oxidation of U(IV) (Stoliker et

al., 2013). This is of concern in the present study, as any oxidation of U(IV) during the

extractions could potentially induce isotopic fractionation and cause the isotopic results

to be invalid. The extraction method used in the present study was modeled on a

technique used by Veeramani et al. (2011), who used a 0.5 M bicarbonate extraction to

successfully determine the amount of adsorbed U(VI) on the surface of biogenic

magnetite. However, Stoliker et al. (2013), reported that bicarbonate extractions, used

to determine the oxidation state of U in aquifer sediments, rapidly oxidized dissolved

U(IV) under anoxic conditions. They also found, under the same conditions, that

minimal oxidation occurred for uraninite. Ginder-Vogel et al. (2006) found that when

high concentrations of $NaHCO_3$ (50 – 100 mM) are present, Fe(III) (hydr)oxides have

the ability to oxidize biogenic uraninite. It should be noted that in this study, Fe(III)

(hydr)oxides are not present and the only oxidant that electrons could be transferred to

is Fe(III) in magnetite. It is not well understood how bicarbonate extractions affect

reactions with U(IV) precipitated on the surface of synthetically made magnetite. So far

studies focusing on bicarbonate extractions to determine U(VI) concentrations

(Veeramani et al., 2011) or induce U(IV) oxidation (Ginder-Vogel et al., 2006; Stoliker et

al., 2013) have used biogenic magnetite or biogenic uraninite. Therefore, it is not

certain if bicarbonate extractions will oxidize U(IV) in experiments using a synthetic

magnetite.

Despite the reports of U(IV) oxidation induced by $NaHCO_3$, observations of the

present study strongly suggest our bicarbonate extractions did not rapidly oxidize U(IV).

First, if bicarbonate extractions were rapidly oxidizing U(IV), after 24 hours of exposure,

we would expect complete recovery of all magnetite-associated U as U(VI). This is not

observed. We also do not see an increasing extracted U(VI) as the reaction progresses and greater masses of U(IV) are present. Furthermore, at the end of the experiments, when most of the dissolved U(VI) was gone, limited recovery of U from the surface of the magnetite occurred (Table 1), indicating that most or all U(IV) must remain on the magnetite. If the bicarbonate bath was rapidly oxidizing U(IV) at the end of the experiment, we would expect extracted U(VI) to match the concentration of the U(VI) lost from solution. The extracted U is much less, indicating that U(IV) remained on the magnetite.

It is possible, however, that the bicarbonate extraction allowed for partial oxidation of U(IV). If oxidation is slow, we could recover some small fraction of the U(IV) during the 24 hour exposure to the bicarbonate bath. The experimental results (concentrations) from this study provide some evidence for this: At the end of both experiments, dissolved U(VI) is almost completely removed from solution, yet we have a small amount of adsorbed U(VI). Using these observations, we compared how the ratio of adsorbed U(VI)/dissolved U(VI) evolved overtime. It appears the adsorbed U(VI) and dissolved U(VI) get close to equilibrium after approximately an hour. We would expect this ratio to remain constant throughout the reaction but this is not that case. At the end, the ratio increases greatly from the trend defined by earlier samples; the concentration of adsorbed U(VI) is much greater relative to the concentration of dissolved U(VI). It thus appears the bicarbonate extractions are recovering a small amount of U(IV), therefore overestimating the amount of U(VI) remaining on the surface of the magnetite, and possibly providing inaccurate isotopic results.

We also considered the possibility that the bicarbonate extraction did not immediately remove adsorbed U(VI) from magnetite surfaces, and that reduction of adsorbed U(VI) continued for some time in the bicarbonate bath. This would cause underestimation of the amount of extracted U(VI), causing the reaction to appear kinetically faster. However we would expect at the much higher (0.5 M) concentrations of $NaHCO_3$ in the bath, desorption of adsorbed U(VI) would be fairly quick, significantly limiting any additional amount of reduction, and the overall underestimation of the amount of extracted U(VI) would be minor.

Overall, the bicarbonate extraction has the potential to alter the true adsorbed U(VI) concentration and calculated U(IV) concentration. In our experiments, we do not see bicarbonate extractions rapidly oxidizing U(IV), but there is the potential for partial oxidation. We expect the underestimation of adsorbed U(VI) due to continued reduction within the bath to be minimal as high concentrations of bicarbonate are known to rapidly desorb U(VI). Based on all of this evidence, we believe the concentrations determined by the bicarbonate extraction to reflect desorbed U(VI) and minimal amounts of oxidized U(IV).

U(VI) Reduction by Magnetite: A Process Model. U(VI) reduction by magnetite is a complex process in which multiple chemical phenomena contribute to the overall reaction. In order for U(VI) reduction to occur, U(VI) in solution must adsorb onto the surface of magnetite. Then, electron transfer from Fe(II) atoms on the surface, and reorganization of U-O bonds must occur to produce U(IV). Unreacted U(VI), and possibly reaction intermediates such as U(V), may desorb from the surface prior to their

conversion to U(IV). This is very important, as three competing processes (reduction, adsorption, or desorption) could potentially induce U isotope fractionation.

In the adsorption process we would expect the uptake of U(VI) in solution to be a net forward reaction (adsorption flux is greater than the desorption flux) when dissolved U(VI) and adsorbed U(VI) concentrations are not in equilibrium. We would expect a minor kinetic isotope effect from the slight bond rearrangement as uranyl groups adsorb. The direction and magnitude is unknown. However, we would expect the magnitude would most likely be small, similar to equilibrium adsorption experiments that observed small U isotope fractionation in published experiments (Brennecka et al., 2011) and in unpublished results from others in our laboratory (N. Jemison, Personal communication).

In the desorption process we would expect a potential for a minor kinetic isotope effect, similar to adsorption, due to the slight bond rearrangement as uranyl groups desorb. The direction and magnitude is unknown, but it is likely the magnitude would also be small.

In the reduction process we expect kinetic effects, in which ^{238}U has a greater rate of reaction than ^{235}U, and ^{238}U is enriched in the product U(IV). The likelihood of such an isotopic fractionation is supported by its occurrence during laboratory microbial U reduction experiments (Basu et al., 2014) and a field-based bioremediation experiment stimulating microbial communities (Bopp et al., 2010). Furthermore, the unusual sense of fractionation, with the heavier isotope reacting faster, is reasonable, given the equilibrium fractionation of about the same magnitude and direction predicted by Schauble (2007). If this same kinetic effect occurs for U(VI) reduction by magnetite,

we would expect [238]U to be removed from the adsorbed U(VI) pool preferentially. This would tend to cause the adsorbed U(VI) pool to become lighter (enriched in [235]U) as reduction proceeded. If desorption and adsorption are in isotopic equilibrium, we would observe the adsorbed U(VI) pool would also be lighter, relative to dissolved U(VI), as reduction proceeded. The dissolved U(VI) and adsorb U(VI) pools would be offset by the isotopic equilibrium fractionation where the dissolved U(VI) pool is ~ 0.2 ‰ heavier (Brennecka et al., 2011; Jemison, personal communication). If the addition and removal of U(VI) from desorption and adsorption were far from equilibrium, we cannot predict the offset, because kinetic effects have not been studied. It is difficult to know if isotopic equilibrium between dissolved and adsorbed U(VI) was approached in our experiments, but this is possible after the first hour or so, given that the concentration of adsorbed U(VI) on the surface of the magnetite and the concentration of dissolved U(VI) decreased together and maintained a nearly constant ratio for the middle part of each experiment.

The overall observed kinetic isotope effect for the overall U(VI) reduction process depends on the relative rates of the individual steps. To understand the overall process we present two extreme cases, where one step is rate-limiting in each case. In the first extreme case, the time scale for reduction of the adsorbed U(VI) pool is very short compared to the time scale for adsorption and desorption. The adsorption step is the rate-limiting step for the overall reduction process, and the amount of U(VI) adsorbed is very small.   Accordingly, the U(VI) desorption flux is very small, and, although the adsorbed U(VI) may be depleted in [238]U because of the ongoing reduction process, the dissolved U(VI) pool is affected little because the desorption flux is so small. The

overall isotope fractionation observed for the overall U(VI) reduction process is thus equal to the adsorption step alone. This conforms to the general rule that in a multi-step process, isotopic fractionation induced by the overall process is equal to the sum of the isotopic fractionation effects for all steps up to and including the rate-limiting step (Basu and Johnson, 2012; Basu et al., 2014; Brunner and Bernasconi, 2005; Hayes, 2001; Johnson, 2012). The adsorption step would be dominantly in the forward direction, with the direction and magnitude of the kinetic isotope effect unknown.

In the second extreme case, the time scale for reduction is much longer than the time scale for adsorption and desorption. In this case, all three processes will affect the adsorbed U(VI) pool. The adsorption/desorption process will be in isotopic equilibrium with U(VI) in solution; we would expect the $\delta^{238}U$ of the remaining U(VI) in solution to be enriched in ^{238}U by about 0.2‰ compared to the adsorbed U(VI) pool. Currently, two studies have observed that adsorption of U causes fractionation of ~ 0.2‰ in $\delta^{238}U$ where the fractionation is constant overtime due to an equilibrium isotope effect (Brennecka et al., 2011; Jemison, Personal communication). If reduction involves a kinetic effect like that observed for microbial reduction, the adsorbed U(VI) pool would become depleted in ^{238}U, which reacts more readily as U(VI) is reduced. The remaining U(VI) in solution and the adsorbed U(VI) pool, linked by isotopic equilibrium, would both become progressively lighter as reduction proceeded.

In experiments 1 and 2, we observed that $\delta^{238}U$ for the adsorbed U(VI) pool did not significantly change as reduction proceeded. However, observed $\delta^{238}U$ values for experiment 1 were always greater than those of experiment 2 (Fig. 2, 4). Experiment 1 was slightly enriched in ^{238}U whereas experiment 2 was enriched in ^{235}U with a

difference in $\delta^{238}U$ ranging from 0.14 ‰ to 0.37 ‰. The experiments also differed in

$\delta^{238}U$ for dissolved U(VI). Experiment 1 observed no shift in $\delta^{238}U$ over time whereas

experiment 2 fluctuated with time. $\delta^{238}U$ was light (~ - 0.12 ‰) but when dissolved

U(VI) was almost completely removed, $\delta^{238}U$ became heavy (~ 0.12 ‰). The

differences between the two experiments were not expected as both reaction conditions

were the same except for increased surface area in experiment 2. Due to the

differences in $\delta^{238}U$ for the adsorbed and dissolved U(VI), surface area of the

magnetite, adsorption/desorption, and reduction, are all contributing to the reaction

differently. If only one of the processes governed U isotopes during reduction, we

would expect the same shift in $\delta^{238}U$ regardless of the overall reaction rate. We would

expect similar experiments would generate the same fractionation in $\delta^{238}U$, but this is

not the case. Therefore, it appears that a different individual process is governing U

isotopes for both experiments.

U(VI) Reduction by Magnetite: Quality Control. We considered the possibility that

these systematic patterns in $\delta^{238}U$ were caused by systematic errors introduced during

sample preparation or mass spectrometry. As part of our standard procedure, we

processed two U isotope standard reference materials (CRM-112a and REIMEP 18-A)

alongside experiment samples. Both standards were purified by UTEVA resin at the

same time as experiment samples. CRM-112a was pre-spiked with the $^{233}U/^{236}U$

double spike whereas REIMEP 18-A was spiked using exactly the same procedure as

experimental samples. Both standards were found to be unaltered by sample

purification; results were similar to values reported by Basu et al (2014) and Shiel et al.

(2013). We therefore found no evidence that $\delta^{238}U$ values could have been altered by sample purification to cause the systematic offsets observed.

We also performed a second, thorough quality control check of these samples to examine analysis parameters that could be influenced by instability of the mass spectrometer or spike/sample ratio. During the analysis, adsorbed U(VI) samples from experiment 1 were analyzed immediately after experiment 2 adsorbed U(VI) samples. This included one sample from experiment 1 (sample 1) analyzed directly after two experiment 2 samples (duplicates of samples 4 and 6). The first measurement of samples 4 and 6 for experiment 2 were analyzed a few hours prior. We found no shift in all of the duplicated samples (Table 1) indicating no instability of the mass spectrometer prior to analysis of experiment 1 adsorbed U(VI) samples. Following measurement of the last experiment 1 adsorbed U(VI) sample (sample 6 - duplicate), we analyzed experiment 2 dissolved U(VI) samples beginning with sample 1. Again, duplicated samples were measured a few hours apart and we found no shift in $\delta^{238}U$ (Table 1). Furthermore, to determine if spike/sample ratio had any influence on the consistent offset between experiments 1 and 2, we measured 4 additional REIMEP 18-A standards that were either under or over spiked. We found no shift in the 5 REIMEP 18-A standards with different spike/sample ratios indicating any small deviation from the ideal spike/sample ratio of ~ 28 would not alter $\delta^{238}U$ values. Therefore, analyzed samples were all within normal ranges and there is no evidence for any type of systematic error that could have caused the observed difference between experiments 1 and 2. Accordingly, we assume that our normal uncertainty envelopes are accurate and the observed offsets in the data reflect processes occurring in the experiments.

U(VI) Reduction by Magnetite: Input and Output of U(VI). To understand why no

major fractionation is observed during reduction we need to think about the input and

output of U(VI) to the adsorbed pool from net adsorption (adsorption and desorption)

and reduction, and their relative rates. For both experiments, at the beginning of the

reaction we observed adsorption added more U(VI) to the surface of the magnetite than

reduction consumed. For example, sample 1 of experiment 2 indicated that 4.42 mg/L

of U(VI) had adsorbed to the surface of the magnetite, but only 1.90 mg/L of that

adsorbed U(VI) was reduced (Table 1).   As the reaction continued, reduction consumed

more U(VI), relative to its addition by net adsorption. This is expected, as the remaining

U(VI) pool shrank and resupply of adsorbed U(VI), via adsorption from the solution, was

limited.

The only major known difference between the 2 experiments is the finer particle

size achieved by grinding the magnetite prior to experiment 2. It appears this difference

affected the amount of U(VI) added to surfaces and we infer it also affected the

reduction rate per unit surface area. Overall, the amount of U(VI) adsorbed and U(VI)

reduced is much greater for experiment 2 relative to experiment 1. As the reactions

proceeded, the change overtime between the input of U(VI) from adsorption and the

output from reduction for experiment 2 is greater and faster than experiment 1.

Experiment 1 is much more gradual whereas experiment 2, the change is much faster

due to approximately 80% of dissolved U(VI) being adsorbed within 1 hour while only

50% U(VI) has be reduced. This is quite interesting as we would expect the first order

reduction rate constant to be much faster for experiment 2 than experiment 1. With

more surface area and sites for U(VI) to adsorb, and overall more available Fe(II) at the

surface, the first order reduction rate constant should be significantly faster. If both experiments contain the same magnetite (same Fe(II):Fe(III)), we would expect reactions containing more surface area would remove more U(VI) from solution and reduce more U(VI) to U(IV). However, this is not the case as the two experiments' first order reduction rate constants were almost equal. At the beginning of experiment 2 we observe larger pools of adsorbed U(VI) on the surface of the magnetite.   We would expect with a greater amount of adsorbed U(VI), more U(VI) should have been reduced. Therefore, the only way for the first order reduction rate constants in the overall experiments (not normalized to surface area) to be equal is for the magnetite in experiment 2 to be less reactive. If less available Fe(II) is present at the surface of the magnetite, less U(VI) reduction will occur.

Using these observations, minor differences in $\delta^{238}U$ for dissolved U(VI) and adsorbed U(VI) can be rationalized based on reaction processes. In experiment 1, we observe that $\delta^{238}U$ for adsorbed U(VI) is slightly positive and dissolved U(VI) remains unchanged. Due to the slower overall reaction rate, we hypothesize the adsorption step is the rate-limiting step. This is supported by the smaller difference between input and output of U(VI) from adsorption and reduction determined by the comparison of U(VI) removed from solution and U(VI) reduced at each time point for both experiments. We do not know if adsorption or desorption itself is inducing the observed fractionation, but it appears that net adsorption does not induce isotopic fractionation. It is possible the heavy adsorbed U(VI) values could have been altered during the bicarbonate extractions. Although we observed a difference between dissolved and adsorbed U(VI), both pools did not have major isotope fractionation during reduction.

For experiment 2, we hypothesize the reduction step is the rate-limiting step as the first order reduction rate is much slower than expected, allowing for the adsorption step to be less rate limiting. At the beginning of experiment 2, $\delta^{238}U$ for dissolved U(VI) and adsorbed U(VI) both evolved quickly to slightly negative values (dissolved U(VI): - 0.12 ‰; adsorbed U(VI): - 0.10 ‰). If reduction were inducing a small kinetic fractionation, the adsorbed U(VI) pool would become enriched in ^{235}U, which we observed. Small differences between dissolved U(VI) and adsorbed U(VI) in the beginning of the experiment are most likely due to minor effects associated with preferential desorption of ^{235}U. As the reaction proceeds, the minor difference between the pools becomes close to zero within the uncertainties. This trend is opposite of experiment 1 where minor differences are found in the middle of the reaction. At the end of experiment 2, adsorbed U(VI) did not change from previous time points, however, we observed completely different $\delta^{238}U$ values for dissolved U(VI) where the remaining U(VI) was heavy. Concentrations for dissolved U(VI) were extremely low, with only 0.3% and 0.1% of the initial U(VI) at the 6 and 12 hour time points, respectively. It is possible that, at the end of the reaction, dissolved U(VI) and adsorbed U(VI) are in isotopic equilibrium where the remaining U(VI) in solution is enriched in ^{238}U. However, this interpretation should be used cautiously as the rate of reduction has nearly ceased but is not zero. The low concentrations would significantly decrease the thermodynamic drive and allow the remaining adsorbed and dissolved pools to be in isotopic equilibrium. Although we observed minor differences in $\delta^{238}U$ for experiment 2, we did not observe any major isotopic fractionation during reduction.

Overall, we can't determine the exact causes of the minor isotopic shifts observed. Given the significant role adsorption has in U(VI) reduction by magnetite and that adsorption is known to cause minor isotopic fractionation, we were not surprised minor isotopic shifts were observed in these experiments. Regardless of the minor shifts between the two experiments, as U(VI) is reduced, we do not observe major isotopic fractionation during reduction.

U(VI) Reduction by Magnetite: U Isotope Fractionation. This is the fourth abiotic study in which very little isotopic fractionation and no change in U isotopes over time was observed during reduction (Rademacher et al., 2006; Stirling et al., 2007; Stylo et al., 2014). These observations are significantly different from previously observed shifts during microbial experiments and a large scale bioremediation field experiment (Basu et al., 2014; Bopp et al., 2010). In the Basu et al. (2014) study, a shift of 1-2 ‰ in $\delta^{238}U$ was observed for multiple microbial strain experiments where the remaining U(VI) became increasingly enriched in ^{235}U as the reaction progressed. At the Old Rifle Site, Bopp et al. (2010) observed a 1‰ shift in $\delta^{238}U$ during a microbial stimulation experiment. Initially, it appears that abiotic reduction does not cause a significant shift in U isotopes; however it is not understood why no significant fractionation is observed during the reduction step in experiment 2.

Focusing on the reduction process may be able to shed some light on why microbial reduction induces fractionation whereas FeS, Zn^0, Fe^0, and magnetite does not. The major difference between microbial and abiotic reduction is most abiotic reactions require the initial adsorption of U(VI) onto a solid surface. If the rate of

adsorption is much slower than the rate of reduction, the adsorption step will be rate-limiting and any observed isotopic fractionation will be due to the steps leading up to and including the adsorption step. If reduction induces major isotopic fractionation, it will not be observed.

If the reduction step is rate-limiting, any major isotopic fractionation induced by this step would be observed in the dissolved U(VI). In microbial reduction, we observe major isotopic fractionation whereas initially, abiotic reduction does not. Focusing on the reduction reaction alone, how does electron transfer occur during U(VI) reduction for abiotic reactions? During microbial reactions, the number of electrons that can be transferred in each step is dependent on the type of microbe and its location of enzymes, structure of cell wall, and metabolic pathways created during growth of the cultures (Basu et al., 2014). If two electrons can be transferred at one time and U(VI) can be reduced directly to U(IV), we would expect significant U isotope fractionation due to a change in U bonding environment. A large shift in U isotopes has been observed for multiple laboratory microbial reduction experiments (Basu et al., 2014).

Unlike microbial reactions, during abiotic reactions, specifically with Fe(II), each Fe(II) can only provide one electron. Therefore, we need to know how two electrons are transferred to reduce U(VI) to U(IV). Two studies have identified that a semi-stable U(V) species has been observed during U(VI) reduction by magnetite (Ilton et al., 2010; Renock et al., 2013). Renock et al. (2013) also proposed a specific mechanism for high U concentrations, where a U(V) disproportionation reaction to U(IV) and U(VI) occurs after a charge transfer to form U(V) where reduction in general is dependent on the oxidation of a passivation layer or Fe(II) at the surface. If U(V) is semi-stable, U(VI)

reduction via single electron transfers could be significantly different in terms of isotope fractionation.

Typically, we expect reactions with significant changes in the local bonding environment around U would induce significant U isotope fractionation due to a change in the stiffness of U-O bonds. Two competing mechanisms induce isotopic fractionation; mass dependent fractionation with enrichment of ^{235}U in the product, and nuclear volume fractionation with enrichment of ^{238}U in the product U(IV) for large atomic number elements like U, where the nuclear volume fractionation is considerably larger (Bigeleisen, 1996). It is possible that the process that forms a semi stable U(V) species could allow the nuclear volume fractionation and mass-dependent fractionation to balance one another out. From theoretical predictions (Bigeleisen, 1996; Schauble, 2007), if equilibrium fractionation between U(VI) and U(IV) is attained, ^{238}U will preferentially partition into the lower oxidation state U(IV), due to U(IV) having a lower electron density at the nucleus. However, no studies have looked at the fractionation of U isotopes during formation of U(V). If the single electron transfer and reduction to U(V) is a kinetic process where U(VI) and U(IV) are not in equilibrium, it is possible the continued reduction to U(IV) is allowing more ^{235}U(VI) to be reduced. Therefore, the nuclear volume fractionation would be muted and we would observe a less significant U isotope fractionation during reduction.

Overall, both reaction kinetics and isotopic fractionation are very complex with U, and thus it is difficult to predict isotopic fractionation. Reaction rate, adsorption, desorption, and reduction all contribute to the overall net isotope fractionation. Any minor changes in input and output from net adsorption or reduction of U(VI) significantly

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affects the overall net isotope fractionation. Therefore, as of yet, we do not have reason to believe all abiotic reactions are fundamentally different from microbial ones. However, if it can be demonstrated that all abiotic reduction mechanisms impart little or no isotopic fractionation, U isotope measurements could then provide an indicator of microbial reduction. This hypothesis should be examined in future work.

U(VI) Reduction by Magnetite: U in the Environment. Overall, our observed lack of significant shift in $\delta^{238}U$ during U(VI) reduction by magnetite has implications for U in the environment. U found in natural, low temperature environments should show no indication of reduction based on $\delta^{238}U$ signature if reactions involving magnetite occurred. Similarly, in paleo-environments, changes in U concentration over time will also have limited isotopic signature if U(VI) reduction by magnetite has occurred. Implications for groundwater remediation are quite interesting. Magnetite is common in aquifer settings but also has the ability to be produced as a by-product during biostimulation of iron reducing bacteria (O'Loughlin et al., 2010). From laboratory experiments with natural or synthetic magnetite, we know magnetite can rapidly and completely reduce U(VI). One would expect, in aquifer settings, that magnetite would significantly influence the concentration of U(VI). In order to monitor reduction using the $\delta^{238}U$ approach, isotopic fractionation must be reflected in the dissolved phase as the reduction reaction requires adsorption of U(VI) onto the surface of magnetite. If the reduction step, as discussed above, is not rate-limiting, we will only observe the adsorption step. The lack of isotopic fractionation leads to an inability to determine if magnetite-driven reduction has occurred, using the isotope approach.

Conclusions

In an aqueous Fe(II) experiment containing U(VI), limited chemical reduction occurred. Our results reinforce the conclusions of Du et al. (2011) that U(VI) reduction by aqueous Fe(II) is limited depending on the reaction conditions. Our experiments were designed to be closer to conditions at relevant natural sites whereas previous studies had conditions that focused on thermodynamics rather than natural conditions. We found that under typical groundwater conditions, reduction is quite limited where a maximum of 25% was achieved. Without significant reduction, we were not able to determine if U(VI) reduction by aqueous Fe(II) induced an isotopic fractionation. However, it is likely aqueous Fe(II) will nonetheless act as a U(VI) reducing agent, via its incorporation into minerals like magnetite, green rust, mackinawite (FeS), or siderite.

In experiments using magnetite as a reductant, U(VI) was rapidly and completely reduced. Our results reinforce the conclusion that stoichiometric magnetite can completely reduce U(VI). Furthermore, XANES analysis confirmed reduction, finding 4 reactors, with 1, 3, 8, and 24 hours of incubation, showed strong reduction and no U(VI) remaining on the surface of magnetite.

For the isotopic analysis, overall results show very little isotopic fractionation as reduction proceeded, in both the dissolved and adsorbed pools. The minor fractionation observed could arise from minor kinetic effects related to adsorption and/or reduction. Minor difference between the experiments could have arisen from differences in reaction conditions, and specifically the extent which each step in the reaction process (adsorption or reduction) is rate-limiting.

Our results for the magnetite experiment add a fourth reaction where U isotope fractionation does not occur during abiotic reduction. The three previous abiotic U studies (Rademacher et al., 2006; Stirling et al., 2007; Stylo et al., 2014) and our study contrast with the U isotopic fractionation observed in field and laboratory microbial experiments (Basu et al., 2014; Bopp et al., 2010). It is not well understood why no major isotope fractionation has been observed during U(VI) reduction by the various abiotic mechanisms. As of yet, we do not have reason to believe all abiotic reactions are fundamentally different from microbial ones. But if it can be demonstrated that abiotic reduction always occurs with little or no U isotopic fractionation, then U isotope data should be useful as an indicator of microbial reduction.

Clearly, more work needs to be done, since multiple U(VI) reduction mechanisms induce different amounts of U isotope fractionation. In order to build a more robust understanding of U isotope fractionation, more isotopic studies are needed, especially with abiotic reduction reactions focusing on Fe(II) and S(-II) minerals commonly found in natural settings. Furthermore, we need to understand how all reaction processes influence U isotopes during abiotic reduction. As we have proposed, different reaction conditions may cause different steps of the overall reaction mechanism to be rate-limiting. In order to understand the magnitude of isotopic fractionation observed in the dissolved phase, we must determine how each process (adsorption, desorption, and reduction) affects U isotopes.
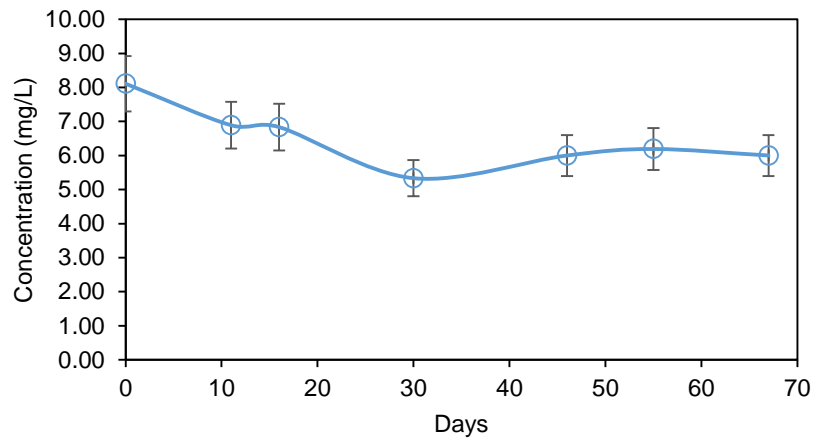
Figures and Tables

Figure 1. Change in U concentration during reduction with aqueous Fe(II). Plot shows reduction of U(VI) over time reaching a maximum % reduced at approximately 25%.
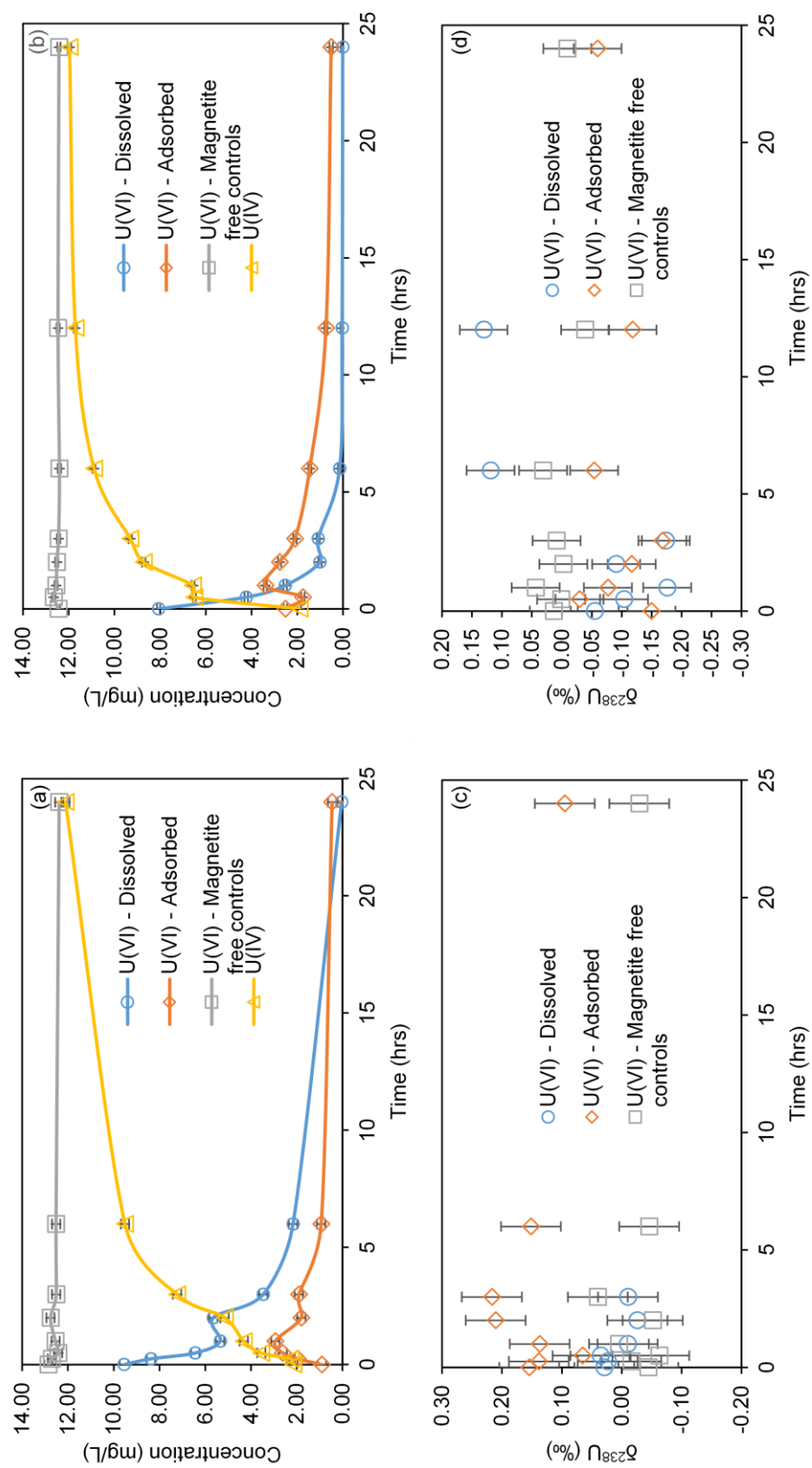
Figure 2. U concentrations and isotope values during U(VI) reduction by magnetite suspensions. Results for experiment 1 are given in (a) and (c); results for experiment 2 are given in (b) and (d). The squares show data from magnetite free control experiments.
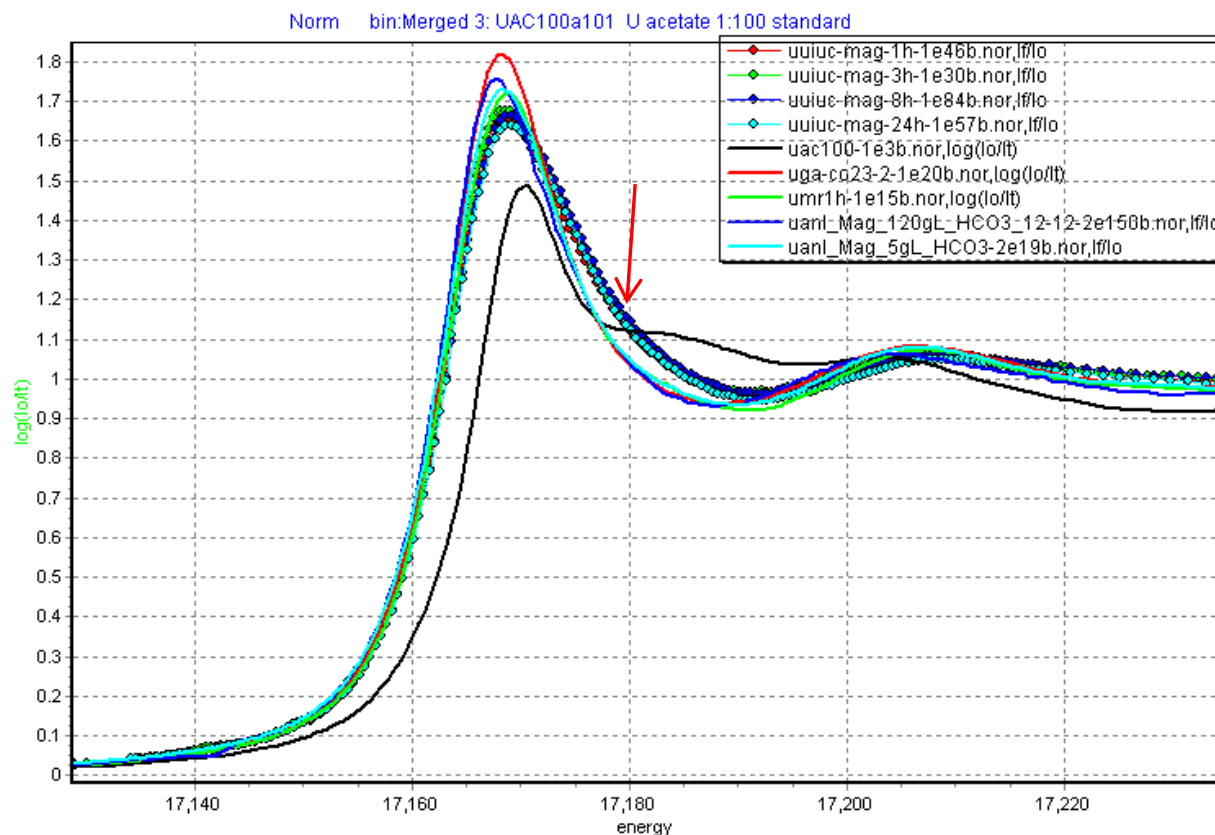
Figure 3. U L$_{III}$-edge XANES data from the U(VI) + magnetite reactions (symbols) compared to a U(VI) standard and various U(IV) standards (biogenic uraninite, U(IV) in magnetite at low and high surface area from (Latta et al., 2014), U(IV)-phosphate from Boyanov et al., (2011) and Fletcher et al., (2010). The edge position of the experiments is similar to that of all U(IV) standards. The experimental samples have greater absorbance than the U(IV) standards in the post edge region, as noted by the arrow. Figure and caption provided by Max Boyanov (Argonne National Lab).
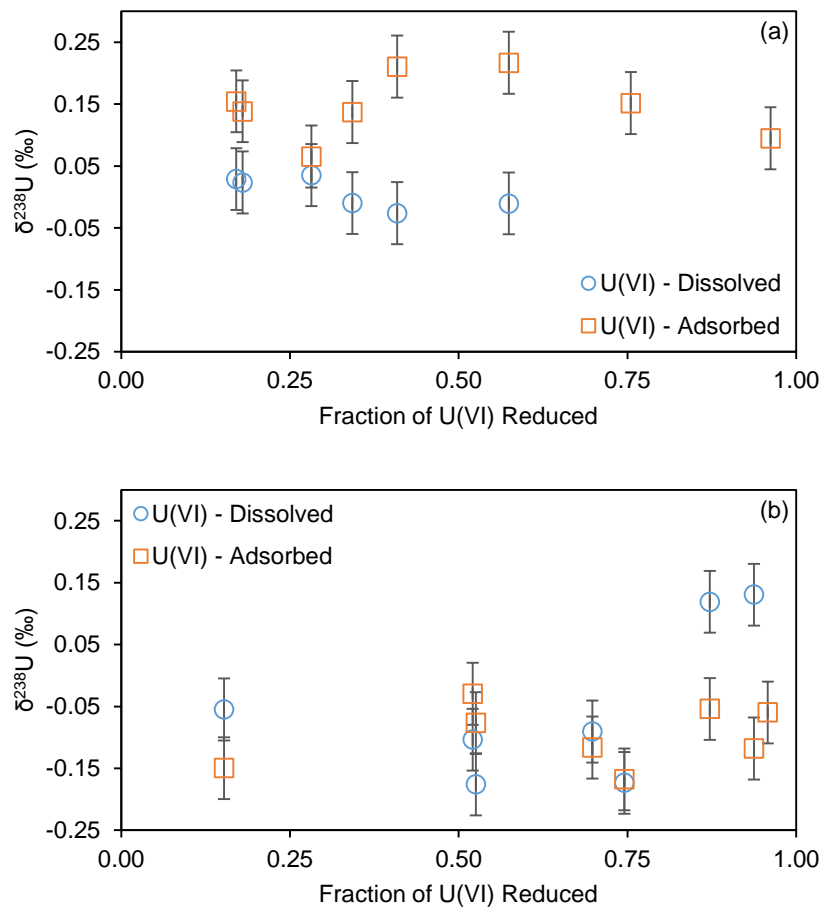
Figure 4. $\delta^{238}U$ values of dissolved U(VI) and adsorbed U(VI) plotted against fraction of U(VI) reduced for U(VI) reduction by magnetite suspensions. Results for experiment 1 are given in (a); results for experiment 2 are given (b).

Table 1. Results from experiments with U(VI) reduction by with magnetite suspensions. Each experiment consisted of eight reactors, each of which was opened at one point in time and processed completely. Magnetite free controls were sampled at each time point and used to determine initial U(VI) concentration, confirm change in concentration was due to reduction, and isotopic fractionation was due to either adsorption or reduction.

| Time (hrs) | U(VI)$_{\text{Magnetite free controls}}$ | | U(VI)$_{\text{Dissolved}}$ | | U(VI)$_{\text{Extracted}}$ | | U(IV) | % Reduction |
	Conc. (mg/L)	$\delta^{238}U$ (‰)	Conc. (mg/L)	$\delta^{238}U$ (‰)	Conc. (mg/L)	$\delta^{238}U$ (‰)	Conc. (mg/L)	
Experiment 1								
0	12.85, 12.60[a]	-0.04, -0.05[a]	9.55, 9.37[a]	0.03, 0.01[a]	0.89	0.15	2.15	17%
0.25	12.71	-0.02	8.37	0.02	1.95, 1.97[a]	0.14, 0.13[a]	2.26	18%
0.5	12.43	-0.06	6.43, 6.52[a]	0.04, -0.01[a]	2.61	0.07	3.55	28%
1	12.55, 12.72[a]	0.00, -0.04[a]	5.35	-0.01	2.93, 2.99[a]	0.14, 0.13[a]	4.31	34%
2	12.76	-0.05	5.65, 5.60[a]	-0.03, 0.02[a]	1.80	0.21	5.15	41%
3	12.52	0.04	3.46	-0.01	1.90, 1.87[a]	0.22, 0.17[a]	7.23	58%
6	12.52, 12.51[a]	-0.05, 0.01[a]	2.16	Not Included[b]	0.93	0.15	9.51	76%
24	12.39	-0.03	0.03	Not Included[b]	0.45	0.09	12.11	97%
Experiment 2 (Finer magnetite)								
0	12.44	0.01	8.07, 8.14[a]	-0.05, -0.07[a]	2.52	-0.15	1.90	15%
0.5	12.65, 12.62[a]	0.00, 0.01[a]	4.23	-0.10	1.75, 1.75[a]	-0.03, -0.05[a]	6.50	52%
1	12.54	0.04	2.53, 2.56[a]	-0.18, -0.18[a]	3.40	-0.08	6.56	53%
2	12.52	0.00	1.00	-0.09	2.77, 2.74[a]	-0.12, -0.11[a]	8.71	70%
3	12.44, 12.43[a]	0.01, -0.02[a]	1.08, 1.07[a]	-0.17, -0.15[a]	2.10	-0.17	9.30	75%
6	12.40	0.03	0.15	0.12	1.45, 1.46[a]	-0.05, -0.04[a]	10.89	88%
12	12.46	-0.04	0.04	0.13	0.74	-0.12	11.70	94%
24	12.42, 12.29[a]	-0.01, 0.05[a]	0.01	Not Included[b]	0.52	-0.06	11.96	96%

a. Duplicate sample preparation and measurement
b. Data not included due to increased error in $\delta^{238}U$ caused by insufficient signal during instrumental analysis

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