Uranium $^{238}U/^{235}U$ Isotope Ratios as Indicators of Reduction: Results from an *in situ* Biostimulation Experiment at Rifle, Colorado, U.S.A.

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The attenuation of groundwater contamination via chemical reaction is traditionally evaluated by monitoring contaminant concentration through time. However, this method can be confounded by common transport processes (e.g. dilution, sorption). Isotopic techniques bypass the limits of concentration methods, and so may provide improved accuracy in determining the extent of reaction. We apply measurements of $^{238}U/^{235}U$ to a U bioremediation field experiment at the Rifle Integrated Field Research Challenge Site in Rifle, Colorado (USA). An array of monitoring and injection wells was installed on a 100 m^2 plot where U(VI) contamination was present in the groundwater. Acetate-amended groundwater was injected along an up-gradient gallery to encourage the growth of dissimilatory metal reducing bacteria (e.g. *Geobacter* species). During amendment, U concentration dropped by an order of magnitude in the experiment plot. We measured $^{238}U/^{235}U$ in samples from one

monitoring well by MC-ICP-MS using a double isotope tracer method. A significant ≈1.00‰ decrease in $^{238}U/^{235}U$ occurred in the groundwater as U(VI) concentration decreased. The relationship between $^{238}U/^{235}U$ and concentration corresponds approximately to a Rayleigh distillation curve with an effective fractionation factor (α) of 1.00046. We attribute the observed U isotope fractionation to a nuclear field shift effect during enzymatic reduction of $U(VI)_{(aq)}$ to $U(IV)_{(s)}$.

Introduction

While the problem of nuclear waste disposal has garnered more public attention, the problems inherent in the front end of the nuclear fuel cycle such as U contamination in surface and groundwaters are also important. Mining and processing of U can pose significant public health hazards, especially if U-rich materials are left exposed at the surface. U production has left a legacy of contamination in many parts of the world (1). Given the possibility of increased reliance on U as an energy source, the issues of contamination and remediation may become more important. Thus, monitoring the remediation of U contamination in groundwater is an important priority for managing the legacy of past U production and the sustainable use of nuclear power.

The environmental chemistry of U resembles that of other redox variable metals and involves a complex combination of adsorption-desorption and redox changes (2,3). U has four naturally occurring oxidation states, ranging from U(III) to U(VI); however U(III) and U(V) are uncommon. U(VI) may be highly mobile in oxic groundwaters under most conditions as the uranyl ion $(UO_2)^{+2}$, particularly when complexed (4). U(IV), by contrast, is highly immobile in all but the most extreme pH conditions. U(IV) forms numerous minerals such as uraninite (5), saturating at low aqueous concentrations. All forms of U are potentially toxic, but reduction of U(VI) to immobile U(IV) greatly reduces the likelihood of exposure via drinking water. Elevated U concentrations are present naturally in felsic igneous bodies such as silicic volcanic rocks or granite (≈4ppm U in average felsic igneous rocks vs. ≈2.5ppm average U content in the earth's upper crust (A)), and may be naturally elevated in watersheds containing these rocks. Economically significant deposits of U in igneous provinces are generally the

result of hydrothermal action, while deposits in sedimentary regimes generally result from the chemical reduction of dissolved $U(VI)_{(aq)}$ in groundwater (6).

U mining and milling has produced almost one million cubic meters of tailings around the world (1) which are most often stored in subaerial piles near the mine or mill which produced them. These tailings piles pose a risk to groundwater because oxidation produces U(VI) that can be mobilized by drainage and enter groundwater. U has been recognized as toxic independent of its radioactivity (7,8), and groundwater is a likely vector for the delivery of U to populations that rely on impacted water supplies. U could also enter the subsurface by infiltration of oxidized waters to the lower parts of the pile or as a component of acidified drainage coming off the tailings pile (1). For a more complete review of the dangers and environmental impacts of U tailings, see (1).

The two most abundant isotopes of U are ^{238}U and ^{235}U, which make up ~99.2% and ≈0.7% of natural U, respectively. Because these two isotopes have extremely long half-lives (≈$4.45*10^9$ and ≈$7*10^8$ years; (9)), they can be treated as "stable" over the relatively short decade-long timescales of processes such as contaminant migration. Recent research demonstrates that on these short timescales, changes in $^{238}U/^{235}U$ are induced by certain geochemical processes, such as chemical reduction. Several authors have shown $^{238}U/^{235}U$ to vary depending on environment and mode of U deposition (i.e. high vs. low temperature or igneous vs. epigenetic sedimentary deposition). These include studies showing broad changes in $^{238}U/^{235}U$ in U-bearing solid materials across several environments, with higher $^{238}U/^{235}U$ occurring in chemically reduced black shales (10,11) and in epigenetic U ores produced by reductive entrapment of $U(VI)_{(aq)}$ from groundwater (12); this latter study found that tabular sandstone ore deposits were consistently depleted in ^{235}U relative to magmatic depostis (12). More recent work by Brennecka, *et al.* (B) shows the same bimodal distribution in ^{235}U content in a larger sample set. Because different isotopes may have slightly different bonding behavior in a given chemical reaction, isotope ratios may be fractionated during chemical reaction. The fractionation of ^{235}U from ^{238}U as a

result of reduction is analogous to the behavior observed in other redox-active elements, such as S (13,14) and Cr (15). Here, we apply the method of using variations in $^{238}U/^{235}U$ to monitor U reduction in a contaminated aquifer undergoing stimulated bioremediation through organic carbon amendment.

Experimental Design

To test the feasibility of *in* situ methods of remediating U contamination in groundwater, an experimental plot was built on the former site of a U mill at Rifle, Colorado (USA). The experiments at this site (the Rifle Integrated Field Research Challenge (IFRC) site) have succeeded in reducing the previously elevated U concentrations by an order of magnitude via stimulation of the native subsurface bacteria (i.e. 16,17). This site is designated the Old Rifle site and was part of the Uranium Mill Tailings Remedial Action (UMTRA) program under the U.S. Department of Energy (17).

We have undertaken the analysis of a set of groundwater samples collected both prior to and during a biostimulated U(VI) reduction experiment. A map of the site is given in figure 1 with the wells studied here noted in bold. The experiment was conducted within a shallow, unconfined aquifer comprised of alluvial sands, silts, and gravels deposited along the Colorado River floodplain and underlain by the impermeable Wasatch Formation at approximately 6 meters depth (17). Groundwater concentrations of $U(VI)_{(aq)}$ in the test plot prior to any groundwater amendment varied from ≈0.42μM to ≈0.95μM, dissolved oxygen was ≈0.2mg/L, and no nitrate was reported.

The "Winchester" field experiment involved the injection of groundwater amended with sodium acetate (5mM) and potassium bromide (2mM) into the subsurface over 31 days during August and September of 2007 to stimulate the bioreduction of U in a manner similar to previous experiments at the site (i.e. 17). Acetate is a common electron donor, and so amending the groundwater with acetate stimulates the activity of dissimilatory metal-reducing microbes. Potassium bromide is a conservative chemical tracer injected with the acetate allowing the flow of amended groundwater to be observed. The

experimental plot (Figure 1) consisted of an injection gallery composed of 10 closely spaced (1 m) injection wells and a larger grid of 12 down-gradient observation wells; three control wells were located up-gradient from the injection gallery. Three rows of down-gradient wells were located 2.5, 5, and 8.5 m from the region of injection; each row was comprised of 4 wells spaced at 2.5 m. The observation wells were constructed of PVC (10 cm diameter) and slotted beneath the water table (ca. 3 m below ground surface at the time of the experiment). The control wells were the same as the experiment wells but located up-gradient of the acetate injection gallery (Figure 1) and were not disturbed except for sampling. This study focuses on a time series of samples taken from well D-07 (5.0 m downgradient of the injection) during organic carbon amendment; this series is compared with samples from control well U-01 during the same time period and background samples from all wells prior to organic carbon amendment.

Background samples were taken from every monitoring well before the injection experiment began. Once acetate injection began, the monitoring wells were sampled periodically (Table 1); this study focuses on samples taken over a period of 45 days from the start of the experiment. For this study, ≈30ml samples of groundwater were filtered through 0.25μm PTFE filters, acidified by the addition of 200μL of ultrapure 12N nitric acid, and stored at 4°C to await analysis. The samples were first analyzed by Inductively-Coupled Plasma Mass Spectrometer for cation content at Lawrence Berkeley National Laboratory before being shipped to the Isotope Geochemistry Laboratory at the University of Illinois at Urbana-Champaign. The samples were stored at 4°C continuously, and were at room temperature only when aliquots were removed for processing and analysis.

Analytical Methods

Double-Isotope Tracer. The double-isotope tracer (double spike) is a mixture of ^{233}U and ^{236}U having a known ratio ($^{233}U/^{236}U$ ≈0.146001). The double spike was admixed into each sample or standard prior to chemical separation to correct for instrumental mass fractionation, (i.e. DE).

Correcting for these effects yields a highly precise determination of the original $^{238}U/^{235}U$. The double spike has a $^{235}U/^{236}U$ ratio of ≈0.00202 and a $^{236}U/^{238}U$ ratio of ≈691, such that the contribution to the total ^{235}U and ^{238}U from the spike is small and can be corrected for. Samples were spiked so as to produce a $^{238}U/^{236}U$ of ~3-4. The double spike methods are similar to those in the literature (12,18).

Wet-Chemical Preparation. Based on kinetic phosphorescence analysis (following ATSM D5174) concentration measurements, aliquots of sufficient volume to yield ≈400ng of U were taken from the field samples. The total sample volume consumed for each prepared aliquot varied from ≈2ml to ≈20ml. After adding ≈125ng of double spike U to each sample, the samples were tightly capped and shaken to thoroughly mix the double spike and sample, then uncapped and evaporated to dryness in a clean dry-down box to promote spike-sample equilibration. Several samples were prepared in duplicate as a check on the entire chemical method.

Samples dissolved in 8N nitric acid were loaded onto a column of AG1-X8 anion exchange resin. The columns varied in volume from 2ml to 4ml (based on sample size). Columns and resin were cleaned by ethanol, sodium hydroxide, 1N HCl, and ultrapure (≈18.2MΩ) water rinses; each column and resin bed was cleaned individually. Immediately prior to use, each column was further cleaned with several column volumes of 1N HCl, 8N nitric acid, and ultrapure water. Samples were then loaded and washed with ≈3.5 column volumes of 8N nitric acid, then eluted in ultrapure water and 1N hydrobromic acid. Samples were then evaporated to dryness, and treated with a small quantity of concentrated nitric acid (≈15N) to destroy organic residues from the resin. The samples were then again evaporated to dryness. The chemical methods are similar to those of Bopp, *et al.* (12), Rademacher, *et al*. (18), and Edwards, *et al* (19).

Isotopic Analysis. Isotopic analyses were made on a Nu Instruments HR Multi-Collector Inductively-Coupled Plasma Mass Spectrometer (MC-ICP-MS) with samples introduced using a

desolvating nebulizer (DSN-100). Purified samples were dissolved in ≈0.3N nitric acid and sample concentrations were adjusted to levels that would provide signal intensities of 160 to 180 pA for ^{238}U, ≈3.5 pA ^{233}U, ≈25 pA ^{236}U, and at least 1 pA for ^{235}U. High-precision $^{238}U/^{235}U$ measurements require sufficient intensity of ^{235}U, long data acquisition times (60 ratios requiring ≈10s per ratio), and long baseline measurements (≈total period of integration) to achieve adequate ^{235}U signal to noise ratio. After tuning and adequate warm up time, several sets of standards including IRMM REIMP-18A (hereafter IRMM U-A) and CRM-112A (formerly NBS U960) are run. All isotope ratio measurements are reported relative to IRMM U-A ($^{238}U/^{235}U$ ≈137.8511 (F) , defined to be 0‰) in the standard delta notation (eq. 1). The reported (11) long-term mean offset of CRM-112A from IRMM U-A is $\delta^{238}U_{IRMM\ U\text{-}A}$+0.21‰. Values for these standards measured at the beginning of each session were used as the major check on the relative accuracy of $^{238}U/^{235}U$ measurements within a session. Sample analysis began after adequate instrument stabilization time, when the observed offset matched the reported offset within the uncertainty of the measurement.

$$\delta^{238}U_{IRMM\ U\text{-}A} = \frac{\left((^{238}U/^{235}U)_{Sample} - (^{238}U/^{235}U)_{Standard}\right)}{(^{238}U/^{235}U)_{Standard}} \times 1000$$

Equation 1

Each sample analysis session included a standard run every third or fourth sample, as well as paired runs of IRMM U-A and CRM-112A, as a check of data quality. We analyzed several samples prepared in duplicate as well as performing repeat instrumental analyses of singly processed samples as a check on data quality and consistency. Repeated analysis of the standards over many analysis sessions shows analytical uncertainty to be ±0.13‰ (2σ), and that no systematic drift occurs. The average offset between $\delta^{238}U_{IRMM\ U\text{-}A}$ and CRM-112A is 0.20‰, which is indistinguishable from the expected value of 0.21‰ (11). Analyses of duplicate samples, and repeat analyses of single samples, show a root-mean-

squared deviation of ≈0.07‰ (n=5 pairs). No abundance sensitivity corrections were necessary and procedural blanks constituted less than 0.002% of the normal U load.

Results and Discussion

While there were small differences in the pre-experiment $\delta^{238}U$ of groundwater within the experimental plot, pronounced changes were observed only after biostimulation occurred. Background samples (Figure 2, Table 1) have a mean $\delta^{238}U_{IRMM\ U\text{-}A}$ of 0.26‰ and standard deviation of 0.12‰ (2σ) excluding two outlying data points. We attribute these anomalous points to the presence of a naturally-occurring reducing zone defined by the presence of reduced minerals and organic material (C). In essence, there is a zone of slow natural reduction in the area of the outlier wells, and the observed lower $\delta^{238}U_{IRMM\ U\text{-}A}$ values reflect a combination of partial U reduction in that zone and mixing of partially-reduced groundwaters with unaffected groundwaters, either as a result of *in situ* advection or sampling.

The down-gradient well D-07 showed a significant change in $\delta^{238}U$ values as the experiment progressed (Figure 3) and groundwater U(VI) concentrations decreased from 0.8 to 0.09μM. Well D-07 showed a total $\delta^{238}U$ change of ≈1.0‰, decreasing from pre-injection values of $\delta^{238}U_{IRMM\ U\text{-}A} \approx 0.40$‰ to post-injection values of $\delta^{238}U_{IRMM\ U\text{-}A} \approx -0.60$‰. This decrease was well outside both analytical uncertainty and the background variability of the site.

The results from the up-gradient control well U-01 contrast strongly with the down-gradient experimental well. Over the same time period as the D-07 results, well U-01 showed no significant change in $^{238}U/^{235}U$ ratio with time (Figure 3); U(VI) concentration also remained stable over the experimental period (0.87±0.02μM). The total variation in $\delta^{238}U_{IRMM\ U\text{-}A}$ over the analysis period was ≈0.11‰ (2σ), with a mean of 0.14‰. Further, no significant co-variation was found between $^{238}U/^{235}U$ and U concentration.

We interpreted these results using a mathematical framework developed in earlier work on isotopic fractionation observed during reduction reactions involving sulfur, selenium, iron, and chromium (15,20,21). The Rayleigh distillation model (22) is often used to describe the relationship between isotope ratios and the extent of reaction. The model describes the isotope ratios of reactants and products in a closed system where a reaction showing isotopic preference proceeds in the absence of back-reaction. To a rough approximation, groundwater masses moving through the bioreduction zone can be described using a Rayleigh model. Each groundwater mass is not a closed system because of dispersive mixing, but this effect leads to relatively small errors in interpretations of isotopic data (23). We assume U(IV) does not undergo back-reaction or otherwise interact substantially with U(VI). Since uraninite has been shown to be stabilized by the presence of organic matter generally (i.e. 24), and biogenic uraninite has been shown to be stabilized by the presence of sulfate-reducers specifically (17, 25), this assumption is tenable. The observed change in $\delta^{238}U$ with concentration is in agreement with such a process. The isotope fractionation factor α (see eq. 2) describes the tendency for a given chemical reaction to fractionate isotopes, and the magnitude of that fractionation. Chemical reactions that show no isotopic preference have an α of unity. We obtained an estimate of α for this site by linear regression through the natural logarithms of $^{238}U/^{235}U$ and U concentration as described in (26). The resulting value of α (see eq. 2) is 1.00046, indicating a small preference for heavier isotopes in the products. The best-fit Rayleigh distillation curve based is plotted with our results in figure 4.

$$\alpha = \frac{\left(^{238}U/^{235}U\right)_{Reactants}}{\left(^{238}U/^{235}U\right)_{Products}}$$

Equation 2

These results indicate that ^{238}U has a greater reaction rate than ^{235}U. This sense of fractionation is opposite that observed for the reduction of lighter elements (i.e. Se, Cr (15, 27), S (28)), in which lighter isotopes have greater reaction rates. However, our findings are consistent with results from U isotopic analyses of solid earth materials (10-12) and theoretical work (G). Recent work has shown that U ore deposits created by entrapment of U via U(VI) reduction in aquifer settings (e.g. roll-front or tabular

sandstone deposits) have a distinctly greater $^{238}U/^{235}U$ compared to higher temperature "magmatic" deposits (12, B). This reflects ^{238}U preferentially reducing to form the solid uraninite phase. Thus, the sense of fractionation derived from ore deposits complements the present study in which the $^{238}U/^{235}U$ in coexisting water decreases as reduction proceeds during the experiment.

Fractionation Mechanisms. Isotopic ratios of the light elements are known to be fractionated during chemical reactions, reflecting differences in bond energy that are dependent upon isotopic masses (29). Numerous studies provide clear evidence of this process in nature and link it to quantum-mechanical "zero-point energy" (ZPE) differences (i.e. (30)). During U reduction, the ZPE effect should cause enrichment of the lighter ^{235}U in the reduced U(IV) precipitate provided that U reduction is the rate-limiting step in the process. This assumption is necessary because, in a chemical reaction with several steps, isotope fractionation is controlled by the rate-limiting step. While it is possible that there is some other reaction limiting U precipitation, the abundance of electron donor (in the form of acetate) in this experiment makes it unlikely that this process is donor-limited. Further, observations of reduced U in other contexts (11-12, B) suggest that mass-dependent fractionation is not the dominant isotope fractionating mechanism. However, our observed fractionation sense is opposite to the ZPE effect.

A mass-independent nuclear effect known as the nuclear field shift (NFS; 31-33) has been shown to be important for heavier elements such as mercury, and it has been applied to explain U isotope fractionation in recent studies (10-12). Unlike conventional, mass dependant effects, the NFS is controlled by the shape and volume of the individual isotope nuclei, independently of atomic mass. Theory predicts that the NFS-driven isotopic fractionation of ^{238}U-^{235}U will be 3 times greater and in the opposite sense to that of the conventional effect (31). The NFS effect should lead to the lighter ^{235}U isotope preferentially staying dissolved in the liquid while the U(IV) precipitate preferentially incorporates ^{238}U, consistent with the data presented here.

While our results are consistent with a NFS-dominated scenario, contradictory results from bacterial reduction experiments do exist. Rademacher, *et al.* (18) found that U reduction in laboratory experiments by *Geobacter sulfurreducens* and *Anaeromyxobacter dehalogenans* resulted in the remaining unreduced U(VI) in solution having progressively higher $^{238}U/^{235}U$; while this is in agreement with a mass-dependent fractionation effect, it is opposite to what is observed in every naturally reduced sample thus far measured (both solids and, in the case here, pore fluids/groundwater). The reason for the difference between the fractionation in the laboratory experiments and that observed during natural U reduction is not understood at present. However, the laboratory microcosm experiments were very different from a natural groundwater system. The results of Rademacher, *et al.* (18) suggest that additional experiments are needed to understand the mechanistic controls on biological fractionation of U isotopes in both natural and laboratory environments.

The use of isotopic methods to monitor remediation and confirm that U reduction has occurred offers a significant advantage over other methods that depend on concentration alone. At Rifle, U concentration decreases rapidly once the acetate injection experiment is underway. The temporal relationship with injection is clear and provides independent evidence that U(VI) reduction is occurring. However, confounding factors such as adsorption, desorption, and dilution can cause significant changes in concentration without U reduction actually taking place such that in many field situations, changes in U concentration cannot be uniquely attributed to reduction. Biostimulation experiments like those performed at Rifle could alter the water chemistry such that U(VI) adsorption is enhanced and U(VI) concentration decreases without any actual U(VI) reduction. We expect that adsorption of U(VI) induces little or no isotopic fractionation because the general bonding environment of U should vary little without a valence change. This lack of fractionation is observed with CrO_4^{2-} adsorption (27). Isotopic fractionation will be strongest when contrasts in the local bonding environment are greatest, e.g. when U(VI) is reduced to U(IV). Although U(VI) sorption could alter the U bonding environment somewhat, we expect that the contrast between dissolved and adsorbed U(VI) species will be much

smaller than that between U(VI) and U(IV). Accordingly, we expect any isotopic fractionation induced by sorption to be much smaller than that induced by reduction. Indeed, sorption of uranyl on MnO2 nodules induces only a 0.2 per mil shift (11). While the potential for sorption to induce U isotope fractionation needs further examination, the systematic decrease in $^{238}U/^{235}U$ with time in D-07 is best explained by fractionation during reduction.

A goal of future work is to determine whether isotopic methods could discriminate abiotic (e.g. Fe(II)-mediated; (36)) reduction from biologically-driven reduction of U(VI) in groundwater. The problem of discriminating between biotic and abiotic U reduction in field experiments is intractable without laboratory data. However, improving in the reactive-transport models used to describe U mobility is a critical objective of field experiments, and so continuing investigation of isotopic methods is warranted.

Impacts. Confirmation of U reduction in field experiments is a difficult problem. In a laboratory setting, it is possible to conclusively demonstrate that U reduction is occurring using micro-analytical methods. In a field environment such confirmation is impossible; the occurrence and extent of U reduction must be assumed or inferred from evidence that is often equivocal. While reducing the concentration of $U(VI)_{(aq)}$ is the final objective of a bioremediation scheme, monitoring $^{238}U/^{235}U$ ratios provides important insight into the pathways of U concentration change because they are primarily controlled by U reduction. Thus, U isotope methods provide an important compliment to U concentration measurements: while concentration of $U(VI)_{(aq)}$ determines the ultimate success of a remediation effort, isotope methods provide important insight into why a remediation scheme succeeds or fails. A scaled-up version of the Rifle experiment described here might achieve a significant reduction in $U(VI)_{(aq)}$ concentrations, but without corroborating data it would be difficult to say why said drop occurred; collecting $^{238}U/^{235}U$ ratio data may provide a relatively fast and efficient method of determining the fate of the remediated U.

The finding of U isotopic fractionation at the Rifle IFRC site provides the most direct evidence that U(VI) reduction in a groundwater system is accompanied by isotopic fractionation. Although such fractionation can be inferred from results of previous studies on naturally occurring solids, this field experiment indicates that $^{238}U/^{235}U$ can be used to trace the progress of reduction in a remediation environment. Because the change in isotope ratio occurs simultaneously with decreases in U(VI) concentration, isotopic ratios appear to provide a method for monitoring U reduction. This method complements standard concentration-based approaches that may be confounded by dilution and sorption. Comparison with baseline or pre-remediation $^{238}U/^{235}U$ can quickly reveal if a reduction-based remediation scheme is working. Furthermore, long-term observations of changes in $^{238}U/^{235}U$ may allow for the assessment of U reoxidation and the potential for remobilization following cessation of organic carbon amendment and a return to more oxic geochemical conditions. It is important to note, however, that this isotopic method cannot be used in settings of anthropogenically altered U isotopic compositions. If the isotopic composition of contaminant U differs from that of the natural environment, then any isotopic signature of reduction will likely be overwhelmed by isotopic changes due to mixing. The method described here may also enable the estimation of very low rates of natural bioreduction occurring on year or decadal timescales.

Acknowledgements

We would like to thank Zhaofeng Zhang and Xiaoxiao Li for their invaluable assistance in the laboratory, and Gideon Bartov for assistance in developing the Rayleigh model. We would also like to thank S. Morris, R. Dayvault, M. Wilkins, A. L. N'Guessan, P. Mouser, and H. Elifantz for their assistance and work on the Winchester experiment. This work was supported in part by NSF grant EAR-0732481 and the Environmental Remediation Science Program, Office of Biological and Environmental Research, U.S. Department of Energy. Lawrence Berkeley National Laboratory is operated for the U.S. Department of Energy by the University of California under contract DE-AC02-

05CH11231. Field experiments were conducted as part of the Integrated Field Research Challenge site at Rifle, CO, USA; a multidisciplinary, multi-institutional project managed for ERSP by Pacific Northwest National Laboratory (PNNL). PNNL is operated for the Department of Energy by Battelle Memorial Institute under contract DE-AC06-76RLO 1830.

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Figure Captions

Figure 1: Map of the experiment plot. Control well U-01, and experiment well D-07, are noted in bold. Direction of groundwater flow is noted by the arrows in the plot, flowing from top to bottom.

Figure 2: Background U isotope measurements across the entire experimental plot. Note the two outlying low values (wells D-03 and D-04; see figure 1) which are attributed to a pre-existing naturally reducing zone in the experiment area. Without the two outliers, the average background $\delta^{238}U$ value is 0.26‰ ±0.12‰ (2σ) indicated by the hatched area. 2σ uncertainty is ≈0.13‰; represented by the error bars shown.

Figure 3: Measured $^{238}U/^{235}U$ isotope ratios in wells D-07 and U-01 over the course of acetate amendment. Well D-07 is down-gradient of the acetate injection gallery, while well U-01 is up-gradient of the injection gallery. $\delta^{238}U$ values in D-07 drop precipitously shortly after acetate amendment begins by ≈1.0‰; while no significant change in U isotope ratio is observed in control well U-01. Uncertainty is ≈0.13‰ (2σ), represented by the error bars; the x-axis indicates days since acetate amendment began.

Figure 4: Observed $^{238}U/^{235}U$ ratios vs. those predicted by a Rayleigh Distillation Model based on the present results; plotted against dissolved U concentration. Reduction is increasing from right to left. The ε for this distillation is ≈0.46‰, computed from the α value as found using the methods in (26). 2σ uncertainty is ≈0.13‰.

Table 1: Results of Uranium Isotope Analysis for selected wells at the Winchester Biostimulation Experiment, Rifle, CO

Well Name/Number	Date	Experiment Elapsed Time (Days)	U Concentration (μM)†	$^{238}U/^{235}U$ $\delta^{238}U_{IRMM\ REIMP-18A}$ (‰)‡
		Background Wells		
D-01	7/29/2009	n/a	0.65	0.30
D-01	7/29/2009	n/a	0.65	0.30
D-02	7/28/2009	n/a	0.43	0.38
D-02	7/28/2009	n/a	0.43	0.29
D-03	7/29/2009	n/a	0.65	0.00
D-04	7/29/2009	n/a	0.64	-0.05
D-05	7/29/2009	n/a	0.73	0.23
D-06	7/28/2009	n/a	0.73	0.19
D-06	7/28/2009	n/a	0.73	0.21
D-07	7/29/2009	n/a	0.64	0.20
D-08	7/29/2009	n/a	0.60	*ND**
D-09	7/29/2009	n/a	0.80	0.22
D-10	7/28/2009	n/a	0.79	0.21
D-11	7/29/2009	n/a	0.76	0.25
D-12	7/29/2009	n/a	0.65	0.16
U-01	7/29/2009	n/a	0.74	0.28
U-02	7/28/2009	n/a	0.55	0.34
U-03	7/29/2009	n/a	0.75	0.32
MNA-1	8/6/2009	n/a	0.95	0.15
		U-01 Timeseries (Upgradient Control Well)		
U-01	8/10/2009	2.00	0.87	0.23
U-01	8/12/2009	4.00	0.89	0.18
U-01	8/14/2009	6.00	0.82	0.17
U-01	8/20/2009	10.00	0.84	0.13
U-01	8/25/2009	15.00	0.87	0.17
U-01	8/29/2009	19.00	0.85	*ND*
U-01	9/1/2009	24.00	0.86	0.20
U-01	9/3/2009	27.00	0.87	0.05
U-01	9/14/2009	37.00	*ND*	0.20
		D-07 Timeseries (Downgradient Experimental Well)		
D-07	8/10/2009	2	0.83	0.38
D-07	8/12/2009	4	0.73	0.41
D-07	8/14/2009	6	0.72	0.49
D-07	8/15/2009	7	0.75	0.36
D-07	8/15/2009	7	0.75	0.32
D-07	8/16/2009	8	0.71	0.32
D-07	8/18/2009	10	0.79	0.23
D-07	8/18/2009	10	0.79	0.34
D-07	8/22/2009	14	0.46	-0.13
D-07	8/23/2009	15	0.38	-0.06
D-07	8/26/2009	18	0.21	-0.62
D-07	8/27/2009	19	0.19	-0.49
D-07	8/28/2009	20	0.15	-0.59

D-07	8/29/2009	21	0.15	-0.54
D-07	8/31/2009	23	0.13	-0.55
D-07	9/3/2009	26	0.10	-0.66
D-07	9/5/2009	28	0.93	-0.61
D-07	9/12/2009	35	0.10	-0.67
D-07	9/13/2009	36	*ND*	-0.64
D-07	9/18/2009	41	0.17	-0.49

*No Data

†± (2σ)

‡ ±0.13‰ (2σ)

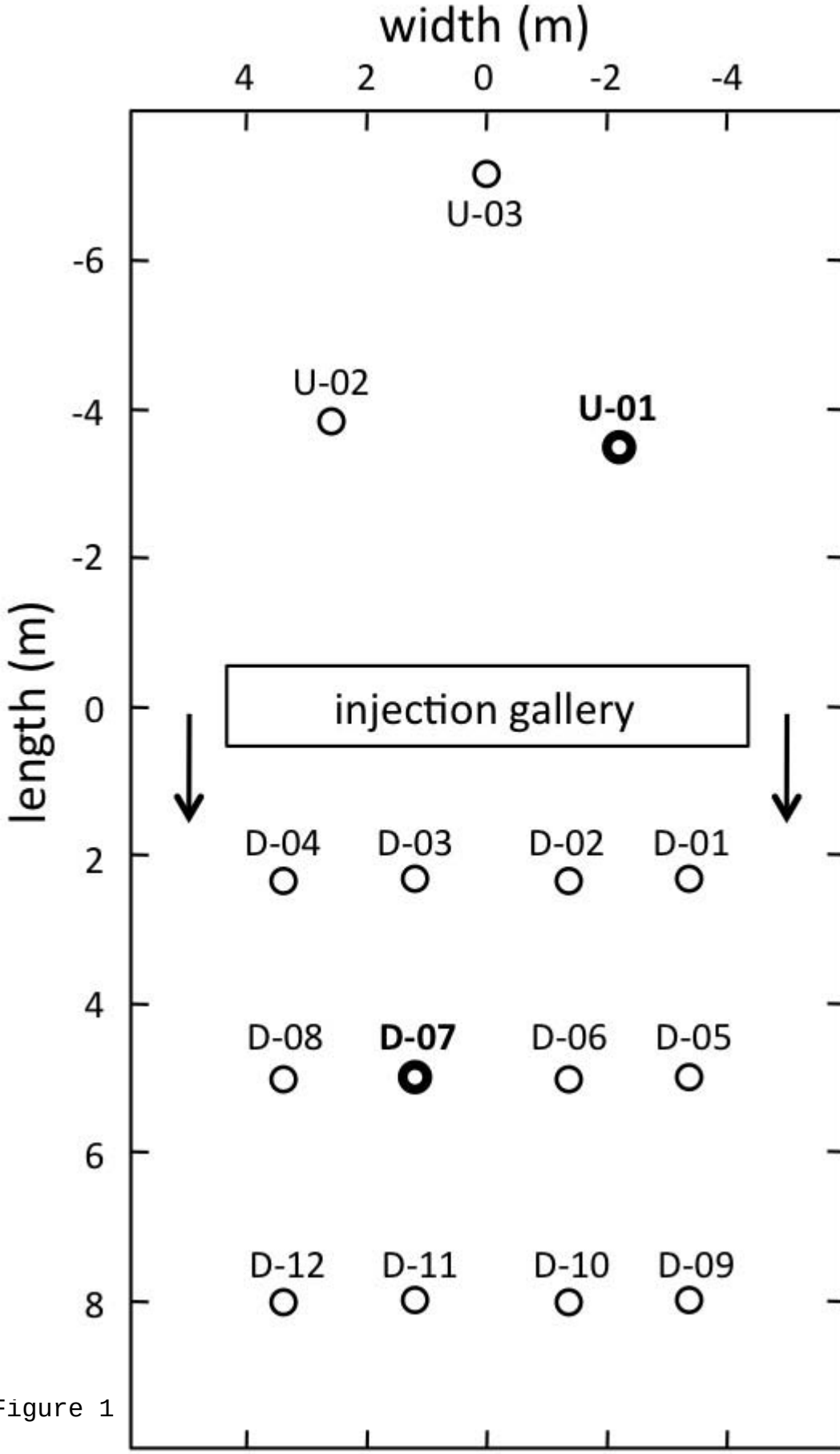
width (m)
4
2
0
-2
-4
length (m)
-6
-4
-2
0
2
4
6
8
U-03
U-02
U-01
injection gallery
D-04
D-03
D-02
D-01
D-08
D-07
D-06
D-05
D-12
D-11
D-10
D-09

Figure 1

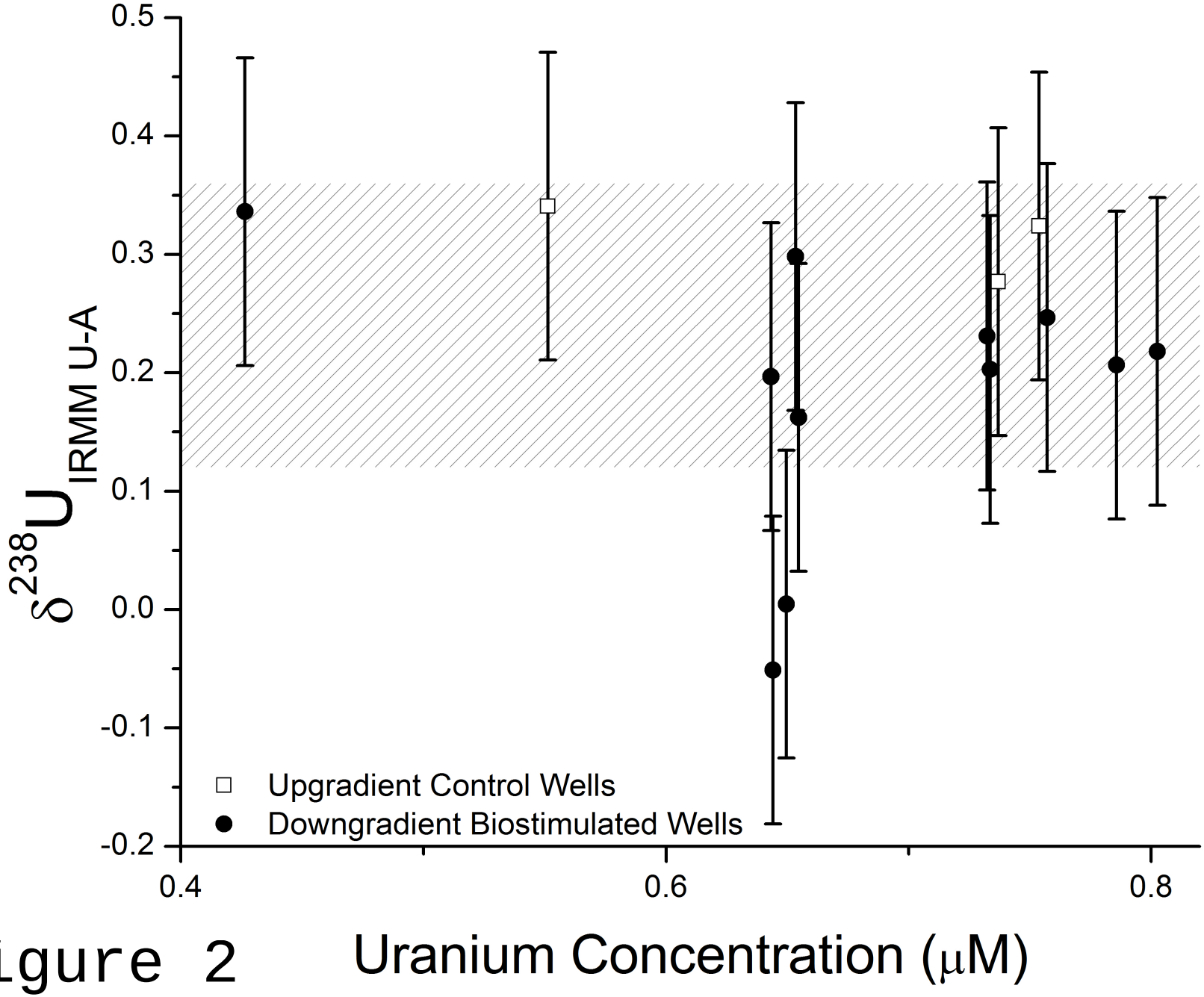
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
$\delta^{238}U_{IRMM\ U\text{-}A}$
Upgradient Control Wells
Downgradient Biostimulated Wells
0.4
0.6
0.8
Uranium Concentration (μM)

Figure 2

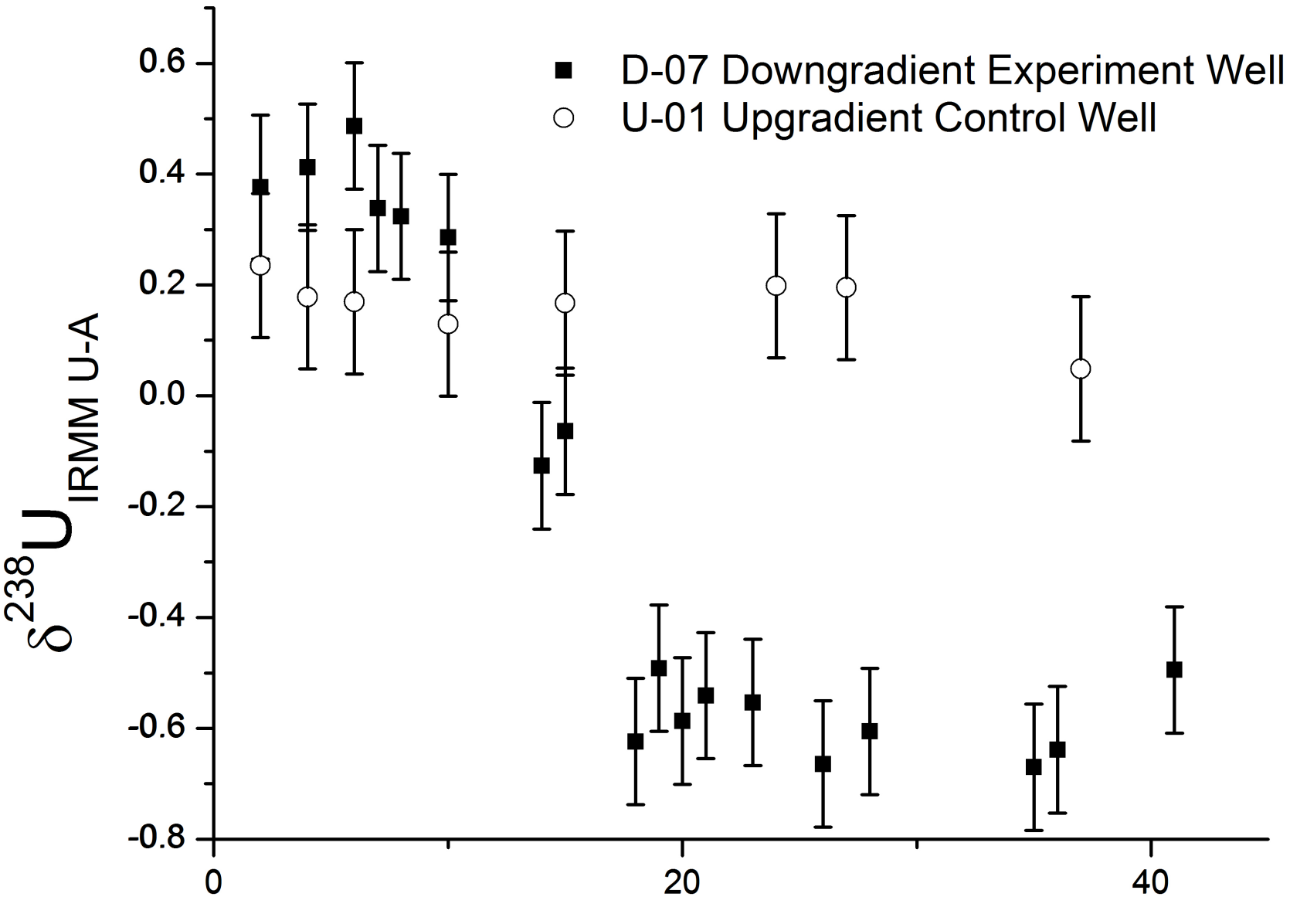
D-07 Downgradient Experiment Well
U-01 Upgradient Control Well
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
$\delta^{238}U_{IRMM\ U\text{-}A}$
0
20
40
Experiment Duration (days)

Figure 3

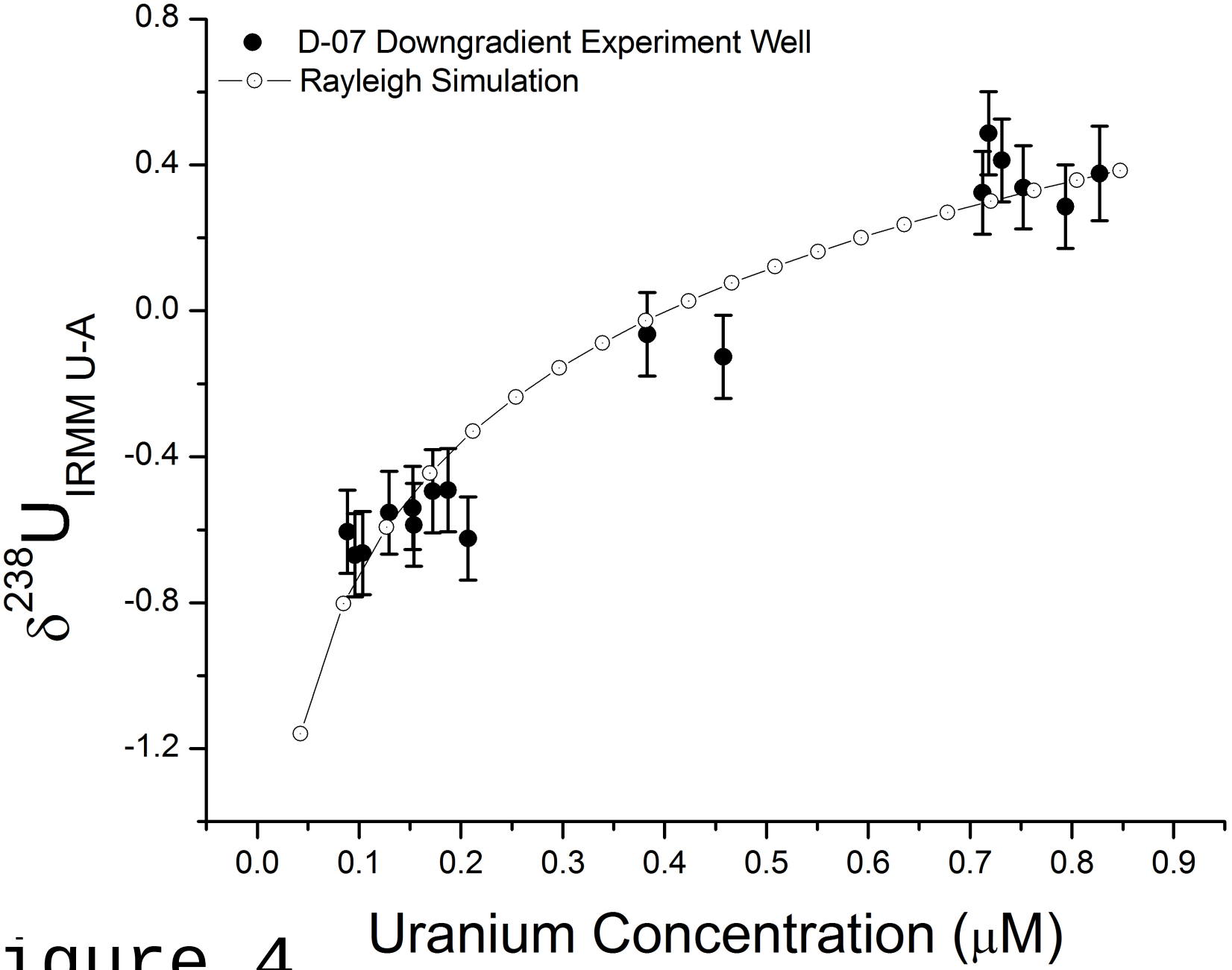
D-07 Downgradient Experiment Well
Rayleigh Simulation
0.8
0.4
0.0
-0.4
-0.8
-1.2
$\delta^{238}U_{IRMM\ U\text{-}A}$
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Uranium Concentration (μM)

Figure 4