

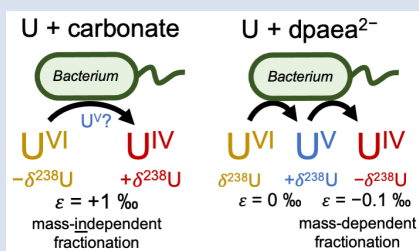
The isotopic signature of U^V during bacterial reduction

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<https://doi.org/10.7185/geochemlet.2411>

Abstract



The two step electron transfer during bacterial reduction of U^{VI} to U^{IV} is typically accompanied by mass-independent fractionation of the ²³⁸U and ²³⁵U isotopes, whereby the heavy isotope accumulates in the reduced product. However, the role of the U^V intermediate in the fractionation mechanism is unresolved due to the challenges associated with its chemical stability. Here, we employed the U^V stabilising ligand, dpaea²⁻, to trap aqueous U^V during U^{VI} reduction by *Shewanella oneidensis*. Whilst the first reduction step from U^{VI} to U^V displayed negligible fractionation, reduction of U^V to U^{IV} revealed mass-dependent isotope fractionation (preferential reduction of the ²³⁵U), contrary to most previous observations. This surprising behav-

our highlights the control that the U-coordinating ligand exerts over the balance between reactant U supply, electron transfer rate, and U^{IV} product sequestration, suggesting that U^V speciation should be considered when using U isotope ratios to reconstruct environmental redox conditions.

Received 13 July 2023 | Accepted 1 March 2024 | Published xx April 2024

Introduction

Hexavalent uranium (U^{VI}) is the predominant oxidation state of U under ambient oxic conditions at Earth's surface and forms soluble uranyl complexes. Under anoxic conditions, reduction of U^{VI} to tetravalent U (U^{IV}) can be mediated by an array of microorganisms or abiotically *via* Fe(II)- or sulfide-bearing compounds (Basu *et al.*, 2014; Brown *et al.*, 2018), resulting in the precipitation of sparingly soluble U^{IV} species. This behaviour has been harnessed for the (bio)remediation of U contaminated groundwater.

Such U redox transformations are often accompanied by changes in the ²³⁸U/²³⁵U ratio, reported as $\delta^{238}\text{U}$ (Andersen *et al.*, 2017). Both *ab initio* calculations and isotope exchange experiments indicate that, at equilibrium, heavy ²³⁸U is enriched in the U^{IV} oxidation state (Schauble, 2007; Abe *et al.*, 2008; Wang *et al.*, 2015). This mass-independent fractionation arises from the nuclear field shift effect (NFSE), due to differences in the size and shape of the nuclei of heavy element isotopologues (Bigeleisen, 1996; Schauble, 2007). At equilibrium, the NFSE is larger than, and operates in the opposite direction to, the conventional mass-dependent isotope effect, whereby the vibrational zero point energy of the lighter isotope leads to its enrichment in U^{IV} as mass-dependent fractionation (MDF) (Bigeleisen, 1996; Schauble, 2007; Fujii *et al.*, 2009). Thus, enrichment of ²³⁸U in U^{IV} following U^{VI} reduction has also been attributed to a dominant NFSE (Weyer *et al.*, 2008; Basu *et al.*, 2014,

2020; Stirling *et al.*, 2015; Stylo *et al.*, 2015), despite not necessarily representing isotopic equilibrium conditions.

As U isotope fractionation is predominantly associated with redox transformations, U isotope signatures have been utilised as a (1) monitoring tool tuned specifically to the reductive rather than adsorptive removal of U^{VI} during remediation (Bopp *et al.*, 2010), and (2) palaeo-redox proxy, whereby the preferential reduction of ²³⁸U during marine anoxia is recorded in sedimentary rocks and can be used to reconstruct the pervasiveness of anoxia in past global oceans (Montoya-Pino *et al.*, 2010; Brennecke *et al.*, 2011; Andersen *et al.*, 2017). Hence, it is crucial to constrain the mechanistic underpinnings of U isotope fractionation to improve the reliability of U isotope based redox reconstructions.

One important aspect of the U reduction mechanism is the role of the pentavalent U (U^V) intermediate. Previous studies have focused on the complete reduction of U^{VI} to U^{IV}. However, there is increasing evidence of the stabilisation and persistence of U^V intermediates within abiotic and biological systems (Roberts *et al.*, 2017; Pan *et al.*, 2020).

During microbiological U^{VI} reduction, two distinct mechanisms for the complete reduction to U^{IV} can occur: either *via* disproportionation of two uranyl^{VI} atoms (generating U^{VI} and U^{IV}) (Vettese *et al.*, 2020), or *via* a second biologically mediated electron transfer to U^V (Molinas *et al.*, 2021, 2023). However, due to the challenges associated with the chemical stabilisation and

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separation of U^V , there is a lack of experimental evidence for its isotopic fractionation, and thus its role in the fractionation mechanism remains unresolved.

Ab initio calculations of the equilibrium isotope fractionation factor combined with a multi-step model of biological U^{VI} -carbonate reduction suggests that fractionation factors of up to 1.6 ‰ for the U^{VI} to U^V step and ~ 0.8 ‰ for the U^V to U^{IV} step (a total of ~ 2.4 ‰) may be expected (Sato *et al.*, 2021). However, these values are significantly larger than those observed in nature or experimentally for U^{VI} to U^{IV} reduction, and it is not clear whether and how redox transformations to and from the U^V intermediate are involved in this discrepancy.

The aminocarboxylate ligand $dpaea^{2-}$ ($dpaeaH_2 =$ bis(pyridyl-6-methyl-2-carboxylate)-ethylamine) can be used to precipitate both U^{VI} and U^{IV} whilst maintaining U^V as an aqueous complex at circumneutral pH (Faizova *et al.*, 2018). These properties have allowed the reduction of U^{VI} by *Shewanella oneidensis* to be followed, revealing the potential for the biological reduction of the U^V intermediate, rather than its disproportionation (Molinas *et al.*, 2021, 2023).

Here, we leveraged the characteristics of $dpaea^{2-}$ to trap aqueous U^V and provide direct experimental evidence of the U^V isotope signature during biological reduction by *S. oneidensis*. The observed isotopic fractionation factors were then compared to those predicted for equilibrium both computationally, using *ab initio* calculations, and experimentally, using isotope exchange approaches (see Supplementary Information for details).

Results and Discussion

The overall experimental flow entails the biological reduction of U^{VI} - $dpaea$ to first U^V - $dpaea$ and then of U^V - $dpaea$ to U^{IV} - $dpaea$. The temporal separation of the two steps, made possible by the vastly different reduction rates, allows the investigation of the isotopic fractionation of one step and then the other. Additionally, the equilibrium isotope fractionation factor was calculated *via ab initio* calculations. Finally, to investigate the equilibrium isotopic fractionation of U^V - $dpaea$ and U^{IV} - $dpaea$, a heavy U^V - $dpaea$ was incubated with a light U^{IV} - $dpaea$ and the isotopic exchange probed over time.

First, U^{VI} - $dpaea$ was produced and reduced biologically. We incubated *S. oneidensis* with solid phase $U^{VI}O_2$ - $dpaea$ and observed a rapid decrease in U^{VI} over 24 hr. This was concomitant with an increase in aqueous U (Fig. 1a) comprising

predominantly U^V (Fig. 1b) that was not observed in abiotic controls (Fig. S-1). Acidification of the aqueous U in 4.5 N HCl, in preparation for ion exchange chromatography, led to the detection of approximately equal quantities of U^{VI} and U^{IV} after separation (Fig. S-2), indicative of U^V disproportionation in the acidified preparation. Collectively, these data suggest that the first electron transfer was achieved rapidly, leading to the accumulation of U^V in solution, in agreement with previous studies (Molinas *et al.*, 2021, 2023).

Aqueous U^V reached its maximum after 24 hr, after which the concentration decreased steadily over fifty days, concomitant with an increase in solid phase U^{IV} (Fig. 1a). This suggests that the second electron transfer proceeds much more slowly than the first. Previous work confirms that reduction from U^V to U^{IV} is indeed mediated by electron transfer from *S. oneidensis*, as opposed to U^V disproportionation (Molinas *et al.*, 2021, 2023). It is likely that reduction of $U^{VI}O_2$ - $dpaea$ proceeds *via* dissolution of the solid uranyl VI followed by rapid reduction of aqueous uranyl VI , *i.e.* dissolution is the rate limiting step for the first electron transfer (Molinas *et al.*, 2023).

A slow second electron transfer step (U^V/U^{IV}) is consistent with abiotic reduction by sodium hydrosulfite (Faizova *et al.*, 2020). Cyclic voltammograms of a $U^V O_2$ - $dpaea$ complex at pH 7 did not display a U^V/U^{IV} reduction event, suggesting slow electron transfer kinetics that may be related to required structural re-arrangements for the formation of a tri-nuclear U^{IV} product (Faizova *et al.*, 2018, 2020).

Uranium isotopic fractionation during the first electron transfer from U^{VI} to U^V was investigated with a dedicated incubation of $U^{VI}O_2$ - $dpaea$ (Fig. 2a). Here, the increasing aqueous U showed negligible changes in $\delta^{238}U$, indicating that the U^{VI}/U^V reduction displayed little fractionation (Fig. 2b). Reduction of U^{VI} by a range of bacterial species typically display enrichment of the heavier ^{238}U in the reduced product, consistent with the predictions of NFS theory during equilibrium isotope fractionation (Basu *et al.*, 2014). Indeed, *ab initio* calculation of the expected isotope fractionation factor between the $U^{VI}O_2$ - $dpaea$ and $U^V O_2$ - $dpaea$ at equilibrium gave a value of 0.82–1.60 ‰ (Table S-1), wherein the positive value signals preferential reduction of ^{238}U . Rather, the isotope signatures of the $U^V O_2$ - $dpaea$ observed in the experiment appear consistent with dissolution being the rate limiting step for the first electron transfer, such that U isotope reduction is rapid and quantitative. As dissolution does not involve a redox reaction, the mass-independent isotope fractionation predicted by the NFSE would not be expected.

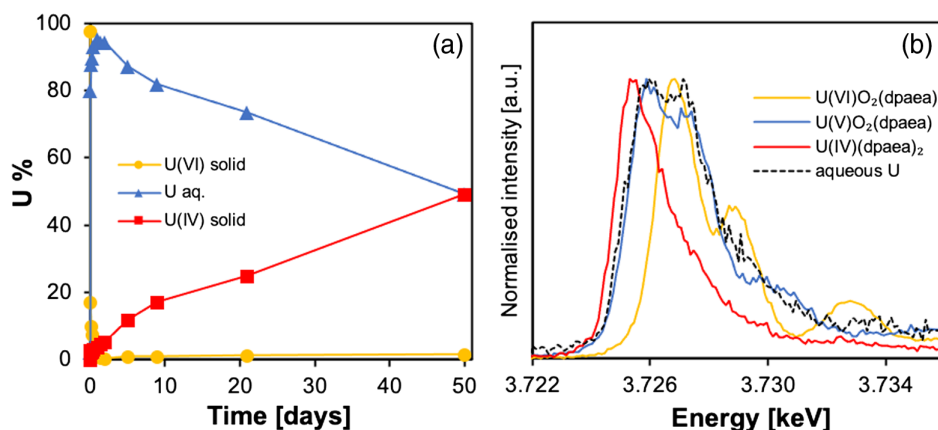


Figure 1 (a) Uranium mass distribution in sacrificial reactors containing *S. oneidensis* incubated with $U^{VI}O_2$ - $dpaea$. (b) Normalised U M_4 -edge HR-XANES spectrum of aqueous uranium after 144 hr of incubation with *S. oneidensis*, along with $U^{VI}O_2$ - $dpaea$, $U^V O_2$ - $dpaea$ and U^{IV} - $(dpaea)_2$ standards.

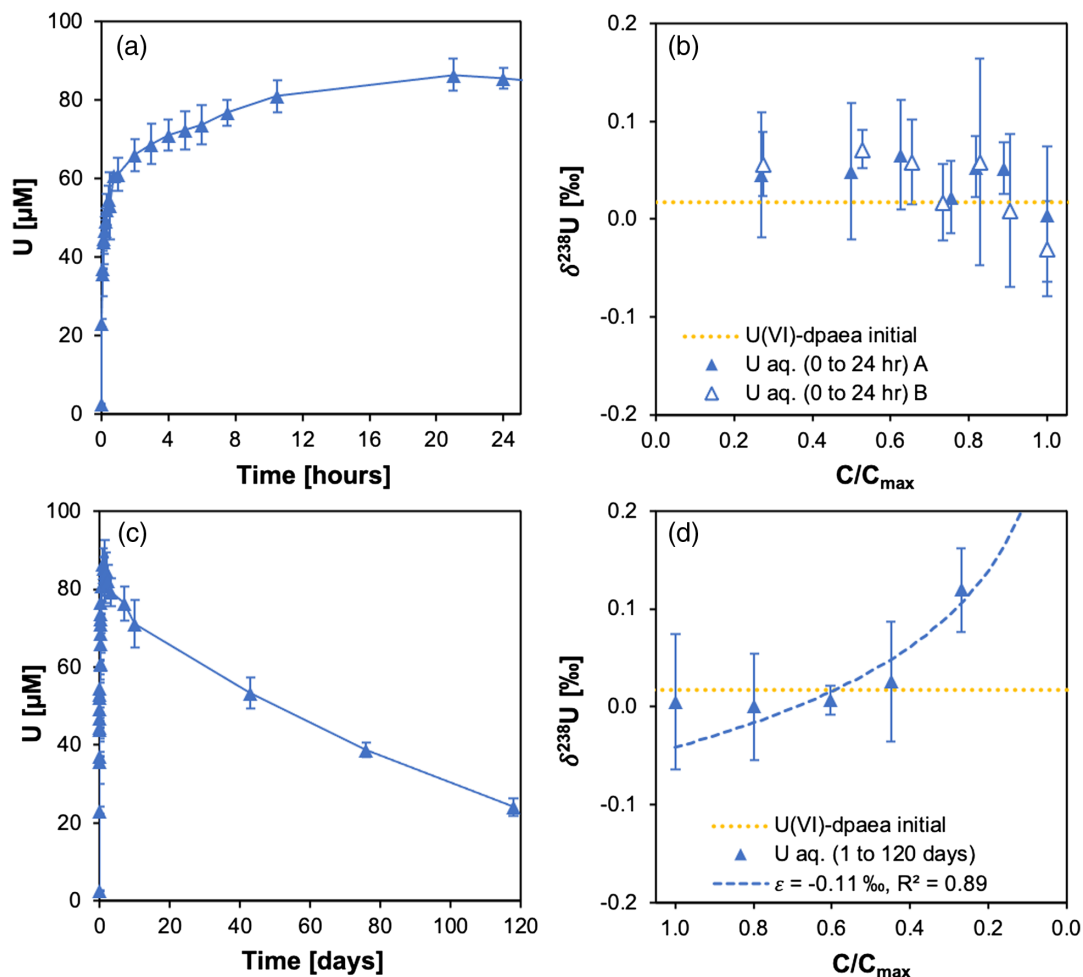


Figure 2 (a) Aqueous uranium concentrations throughout the first 24 hr of incubation of $U^{VI}O_2$ -dpaea and *S. oneidensis*. Symbols and error bars depict one standard deviation of the mean of duplicate reactors. (b) Corresponding $\delta^{238}U$ values of the aqueous U in duplicate systems (A and B), reported as a fraction of the maximum aqueous U concentration. Symbols and error bars depict two standard deviations of the mean of triplicate measurements. The $\delta^{238}U$ value of the initial $U^{VI}O_2$ -dpaea is plotted as a yellow dotted line. (c) Aqueous uranium concentrations throughout the whole reaction between $U^{VI}O_2$ -dpaea and *S. oneidensis*. Symbols and error bars depict one standard deviation of the mean of duplicate reactors. (d) $\delta^{238}U$ values of the aqueous U after 24 hr when the aqueous U concentration began to decrease. Values are reported as a fraction of the maximum aqueous U concentration. Symbols and error bars depict two standard deviations of the mean of triplicate measurements. The Rayleigh model (blue dashed line) corresponds to the linear best fit of the logarithmic data, $R^2 = 0.89$, from which the isotope enrichment factor, ϵ , is derived.

Once U^{VI} was completely reduced and aqueous U^V reached its maximum concentration after 24 hr, the isotope signature of the aqueous U^V was measured to quantify fractionation during the U^V/U^{IV} reduction step (Figs. 2d, S-3). Although limited fractionation was observed, Rayleigh distillation models could be fitted to the data, indicating fractionation factors (ϵ) of -0.10 ‰ and -0.11 ‰ for the two batch replicates. These negative values indicate the preferential accumulation of lighter ^{235}U in the reduced product, contrary to previous observations for microbial U^{VI} reduction and at odds with NFS theory (Basu *et al.*, 2014; Stirling *et al.*, 2015; Stylo *et al.*, 2015). To ascertain whether this direction of fractionation reflected equilibrium in the peculiar case of a strong aminocarboxylate ligand, we performed *ab initio* calculations of the fractionation factor at equilibrium between $U^{VI}O_2$ -dpaea⁻ and either U^{IV} -(dpaea)₂ or a non-uraninite U^{IV} species, the two likely products of this biological reaction (Molinas *et al.*, 2021). We modelled the non-uraninite U^{IV} as a cluster of ningyoite (CaU(PO₄)₂), a close analogue of the non-crystalline biotic reduction products (Bernier-Latmani *et al.*, 2010; Alessi *et al.*, 2014). The fractionation factors of 0.27–0.33 ‰ for the U^{IV} -(dpaea)₂ product and

0.13–0.46 ‰ for ningyoite both reveal that ^{238}U would be enriched in the U^{IV} product at equilibrium (Table S-1), contrary to that observed during biological reduction. These calculations indicate that the bioreduction system was far from equilibrium and suggest that the reaction mechanism precluded the full expression of NFSE that would have enriched ^{238}U in the product. Furthermore, recent work has proposed that slow microbial reduction should impart significant mass-independent fractionation of up to +1 ‰ (Brown *et al.*, 2018; Basu *et al.*, 2020), whereas negative fractionation factors are typically only observed for rapid abiotic reductions, on the order of hours (Stylo *et al.*, 2015). The slow reduction of the $U^{VI}O_2$ -dpaea⁻ observed in our experiments (on the order of months), suggests that the proposed reduction rate-fractionation relationship does not hold for all circumstances.

To investigate whether equilibrium isotope exchange and the associated expression of the NFSE could overprint the reduction-derived MDF signature, we performed isotope exchange experiments between the solid U^{IV} product of the bioreduction experiment, with an initial (light) $\delta^{238}U$ of 0 ‰, and aqueous $U^{VI}O_2$ -dpaea⁻, with an initial (heavy) $\delta^{238}U$ of ~ 5 ‰. Over 200

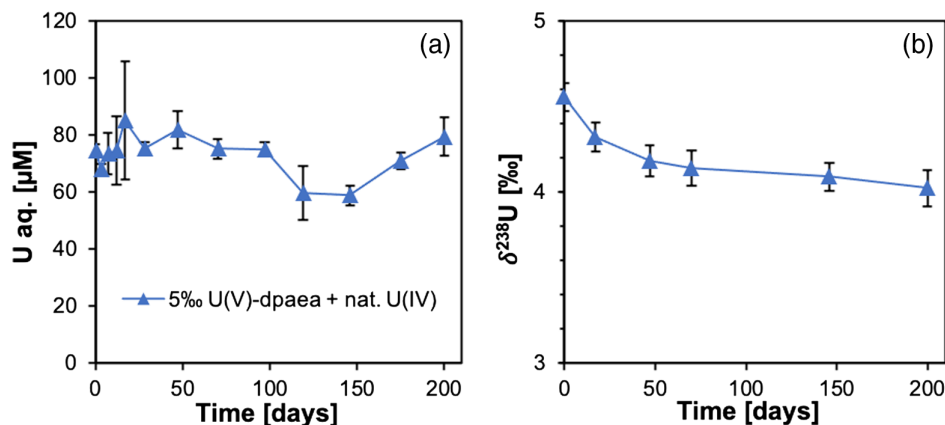


Figure 3 (a) Aqueous uranium concentrations during equilibrium isotope exchange experiments between $U^{VI}O_2\text{-dpaea}^-$ with an initial isotopic composition of $\sim 5\text{‰}$, and U^{IV} present as the product of the bioreduction experiments of natural U, with an initial isotopic composition of 0‰ . Symbols and error bars depict one standard deviation of the mean of duplicate reactors. (b) $\delta^{238}U$ values of the aqueous U. Symbols and error bars depict one standard deviation of the mean of duplicate reactors.

days, aqueous U became isotopically lighter by 0.6‰ , indicating the preferential accumulation of ^{238}U in the U^{IV} solid (Fig. 3). Whilst this direction of fractionation is in agreement with that calculated for equilibrium, isotope mass balance calculations indicate that the U^{IV} solid did not become heavier than the aqueous U^V , contrary to the computed equilibrium. These data show that progress to full equilibrium is significantly limited over the course of the experiment, presumably due to slow ligand exchange kinetics.

This hypothesis is consistent with the strong pentadentate coordination of U^V by dpaea, which provides protection from ligand dissociation and cation-cation interactions typical of U^V disproportionation (Faizova *et al.*, 2018). Furthermore, any preferential re-oxidation of $^{235}U^{IV}$ to U^V would require the *de novo* formation of the two uranyl dioxo bonds and re-coordination with dpaea. This is likely kinetically limited due to steric hinderance by the U^{IV} coordinating ligands. Therefore, we propose that isotope signatures indicating mass-dependent fractionation (faster reaction of ^{235}U) are preserved during the biological reduction of $U^V O_2\text{-dpaea}^-$ because subsequent equilibrium isotope exchange, fractionating in the opposite direction, is limited.

Regardless of the abiotic equilibrium isotope exchange between reactants and products (independent of the bioreduction reaction), a recent model has demonstrated the importance of back reaction within the U^{VI} bioreduction pathway in controlling the overall isotope fractionation (Sato *et al.*, 2021). The model stipulates that the overall isotope fractionation at each reaction step arises from the balance between the forward and backward reaction rates, and the attendant isotope fractionation for the forward and backward reactions. As such, reactions with equal forward and backward reaction rates will display the full fractionation factor predicted for equilibrium (typically positive for U reduction, indicating preferential accumulation of ^{238}U in the product). On the other hand, irreversible reactions will result in no observed fractionation. The theory of this model has been demonstrated experimentally during U^{VI} reduction by *S. oneidensis*, in which back reaction (reverse electron transfer) was limited by large electron fluxes from oxidation of the electron donor (Brown *et al.*, 2023a). These systems result in significantly less isotope fractionation than those with small electron fluxes, which permit more back reaction. The theoretical model and associated experimental evidence, coupled to our observations of the isotope exchange experiment, would suggest that back reaction during biological reduction of $U^V O_2\text{-dpaea}^-$ is limited and point toward

the role of the U coordinating ligand in controlling the magnitude of isotope fractionation.

Furthermore, during microbiological reduction of U^{VI} -carbonate, the conventional isotopic mass effect was fully expressed, while the NFSE was not (Brown *et al.*, 2023b). This implies that the mass-dependent vibrational effect and the mass-independent NFSE are two competing effects operating in opposing directions and is consistent with the proposal that the NFSE requires reaction reversibility in order to overprint the mass-dependent effect.

Collectively, these studies indicate that the inhibition of back reaction in the dpaea system is so severe that the mass-dependent isotope fractionation factor is preserved. More specifically, we propose the following mechanism: first, the flux of electrons from the cell to the outer membrane U-reducing proteins is significantly greater than the U^V reduction rates (limited by either low redox potential and/or steric hinderance) (Fig. 4). This allows the redox-active Fe-bearing heme groups of these proteins to become fully reduced prior to electron transfer to U^V . Eventually, electron transfer from the heme Fe^{II} to U^V occurs with isotopic fractionation according to the conventional mass effect – faster reaction of ^{235}U . Concurrently, a rapid continuous flux of electrons from metabolism re-reduces the Fe^{III} of the

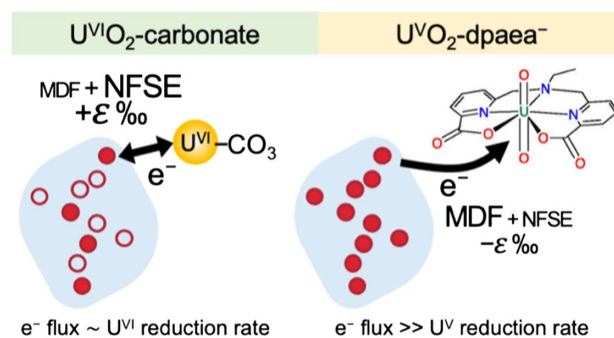


Figure 4 Cartoon of the proposed mechanism of U isotope reduction and fractionation for both U^{VI} -carbonate (left) and $U^V\text{-dpaea}^-$ (right). Electrons are transferred from the cell to outer membrane U reducing proteins (blue areas) containing multiple redox active heme iron centres (red circles). Depending on the flux of electrons, the heme iron centres are either in their reduced state (solid fill) or oxidised state (open fill).

heme group (empty circles in Fig. 4) and prevents reverse electron transfer from the newly reduced U^{IV} . Consequently, isotopic equilibration that is dominated by the mass-independent NFSE cannot over-print the initial MDF, unlike in U-carbonate containing systems.

Likewise, back reaction may also be limited by U^{IV} sequestration, *i.e.* kinetic limitations imposed by the U^{IV} structure and bond rearrangement to recover the uranyl bond structure, resulting in significantly faster electron transfer rates from the heme Fe^{II} to $U^{VO_2-dpaea^-}$ than U^{IV} to heme Fe^{III} .

Conclusions

We employed the U^V stabilising ligand, dpaea, to trap aqueous U^V and observed, for the first time, the isotopic signature of U^V throughout the bioreduction of U^{VI} to U^{IV} . Whilst the observation of a mass-dependent isotope fractionation factor appears to conflict with previous studies of microbial U reduction, this is likely not an artefact of the unique properties of dpaea (*i.e.* its ability to solubilise and trap U^V). Rather, these adventitious properties have elucidated the control U coordinating ligands exert over the balance between reactant U supply, electron transfer rate, and U^{IV} product sequestration. Thus, we infer that other ligands (that cannot stabilise U^V) will behave similarly when such conditions are met. This has significant implications for the interpretation of U isotope signatures in environments where the availability of high affinity ligands may impact U lability. For example, in reducing environments with considerable amounts of organic carbon (providing both a source of electrons for microbial U^{VI} reduction and a supply of organic complexants), the contribution of the NFSE to observed isotopic signatures may be diminished. This may lead to false interpretations of U isotope signatures, *e.g.*, in applications using organic-rich anoxic sediments as a palaeo-redox archive. In such studies, the observation of lower $\delta^{238}U$ (arising from NFSE-dominated mass-independent fractionation) is usually thought to indicate either a local shift in depositional conditions or water column stratification (Andersen *et al.*, 2017; Brüske *et al.*, 2020; Lau *et al.*, 2022), or a shift in the U isotope mass balance, resulting from enhanced oceanic anoxic environments at regional or global scales (Montoya-Pino *et al.*, 2010; Andersen *et al.*, 2017). However, our results show that the extent and direction of U isotope fractionation during U reduction may depend on the stabilisation of U^V and, more generally, the lability of U complexes.

Furthermore, this study suggests that full expression of isotopic equilibrium in nature may be precluded by U speciation, in addition to the previous roles reported for electron flux and U supply dynamics (Basu *et al.*, 2020; Brown *et al.*, 2023a). Future work should focus on delineating these controls with an aim to incorporate U speciation as a parameter within models of U isotope fractionation in the environment.

Acknowledgements

Funding for this work was provided by an ERC consolidator grant awarded to RB-L (725675: UNEARTH: "Uranium isotope fractionation: a novel biosignature to identify microbial metabolism on early Earth"). This work was also supported by JSPS KAKENHI Grant Numbers JP19K22171, JP21H01864 and JP22J12551. A part of the calculations was performed at the Research Center for Computational Science, Okazaki, Japan (Project: 21-IMS-C049 and 22-IMS-C049). Pierre Rossi provided invaluable support in complying with radiological safety regulations in the lab. We also thank two reviewers and the editor, Claudine Stirling, for their constructive input.

Editor: Claudine Stirling

Additional Information

Supplementary Information accompanies this letter at <https://www.geochemicalperspectivesletters.org/article2411>.



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Cite this letter as: Brown, A.R., Molinas, M., Roebbert, Y., Faizova, R., Vitova, T., Sato, A., Hada, M., Abe, M., Mazzanti, M., Weyer, S., Bernier-Latmani, R. (2024) The isotopic signature of U^V during bacterial reduction. *Geochem. Persp. Lett.* 29, 1–6. <https://doi.org/10.7185/geochemlet.2411>

References

- ABE, M., SUZUKI, T., FUJII, Y., HADA, M., HIRAO, K. (2008) An *ab initio* molecular orbital study of the nuclear volume effects in uranium isotope fractionations. *The Journal of Chemical Physics* 129, 164309. <https://doi.org/10.1063/1.2992616>
- ALESSI, D.S., LEZAMA-PACHECO, J.S., STUBBS, J.E., JANOUSCH, M., BARGAR, J.R., PERSSON, P., BERNIER-LATMANI, R. (2014) The product of microbial uranium reduction includes multiple species with U(IV)-phosphate coordination. *Geochimica et Cosmochimica Acta* 131, 115–127. <https://doi.org/10.1016/j.gca.2014.01.005>
- ANDERSEN, M.B., STIRLING, C.H., WEYER, S. (2017) Uranium Isotope Fractionation. *Reviews in Mineralogy and Geochemistry* 82, 799–850. <https://doi.org/10.2138/rmg.2017.82.19>
- BASU, A., SANFORD, R.A., JOHNSON, T.M., LUNDSTROM, C.C., LÖFFLER, F.E. (2014) Uranium isotopic fractionation factors during U(VI) reduction by bacterial isolates. *Geochimica et Cosmochimica Acta* 136, 100–113. <https://doi.org/10.1016/j.gca.2014.02.041>
- BASU, A., WANNER, C., JOHNSON, T.M., LUNDSTROM, C., SANFORD, R.A., SONNENTHAL, E., BOYANOV, M.I., KEMNER, K.M. (2020) Microbial U isotope fractionation depends on U(VI) reduction rate. *Environmental Science and Technology* 54, 2295–2303. <https://doi.org/10.1021/acs.est.9b05935>
- BERNIER-LATMANI, R., VEERAMANI, H., VECCHIA, E.D., JUNIER, P., LEZAMA-PACHECO, J.S., SUVOROVA, E.I., SHARP, J.O., WIGGINTON, N.S., BARGAR, J.R. (2010) Non-uraninite products of microbial U (VI) reduction. *Environmental Science and Technology* 44, 9456–9462. <https://doi.org/10.1021/es101675a>
- BIGEISEN, J. (1996) Nuclear size and shape effects in chemical reactions. Isotope chemistry of the heavy elements. *Journal of the American Chemical Society* 118, 3676–3680. <https://doi.org/10.1021/ja954076k>
- BOPP, C.J., LUNDSTROM, C.C., JOHNSON, T.M., SANDFORD, R.A., LONG, P.E., WILLIAMS, K.H. (2010) Uranium $^{238}U/^{235}U$ isotope ratios as indicators of reduction: Results from an *in situ* biostimulation experiment at Rifle, Colorado, U.S.A. *Environmental Science and Technology* 44, 5927–5933. <https://doi.org/10.1021/es100643v>
- BRENNECKA, G.A., HERRMANN, A.D., ALGEO, T.J., ANBAR, A.D. (2011) Rapid expansion of oceanic anoxia immediately before the end-Permian mass extinction. *Proceedings of the National Academy of Sciences of the United States of America* 108, 17631–17634. <https://doi.org/10.1073/pnas.1106039108>
- BROWN, S.T., BASU, A., DING, X., CHRISTENSEN, J.N., DEPAOLO, D.J. (2018) Uranium isotope fractionation by abiotic reductive precipitation. *Proceedings of the National Academy of Sciences of the United States of America* 115, 8688–8693. <https://doi.org/10.1073/pnas.1805234115>
- BROWN, A.R., MOLINAS, M., ROEBBERT, Y., SATO, A., ABE, M., WEYER, S., BERNIER-LATMANI, R. (2023a) Electron flux is a key determinant of uranium isotope fractionation during bacterial reduction. *Communications Earth and Environment* 4, 329. <https://doi.org/10.1038/s43247-023-00989-x>
- BROWN, A.R., ROEBBERT, Y., SATO, A., HADA, M., ABE, M., WEYER, S., BERNIER-LATMANI, R. (2023b) Contribution of the nuclear field shift to kinetic



- uranium isotope fractionation. *Geochemical Perspectives Letters* 27, 43–47. <https://doi.org/10.7185/geochemlet.2333>
- BRÜSKE, A., WEYER, S., ZHAO, M.Y., PLANAVSKY, N.J., WEGWERTH, A., NEUBERT, N., DELLWIG, O., LAU, K.V., LYONS, T.W. (2020) Correlated molybdenum and uranium isotope signatures in modern anoxic sediments: Implications for their use as paleo-redox proxy. *Geochimica et Cosmochimica Acta* 270, 449–474. <https://doi.org/10.1016/j.gca.2019.11.031>
- FAIZOVA, R., SCOPELLITI, R., CHAUVIN, A.-S., MAZZANTI, M. (2018) Synthesis and characterization of a water stable uranyl(V) complex. *Journal of the American Chemical Society* 140, 13554–13557. <https://doi.org/10.1021/jacs.8b07885>
- FAIZOVA, R., FADAEI-TIRANI, F., BERNIER-LATMANI, R., MAZZANTI, M. (2020) Ligand-supported facile conversion of uranyl(VI) into uranium(IV) in organic and aqueous media. *Angewandte Chemie* 132, 6822–6825. <https://doi.org/10.1002/ange.201916334>
- FUJII, T., MOYNIER, F., ALBARÈDE, F. (2009) The nuclear field shift effect in chemical exchange reactions. *Chemical Geology* 267, 139–156. <https://doi.org/10.1016/j.chemgeo.2009.06.015>
- LAU, K.V., HANCOCK, L.G., SEVERMANN, S., KUZMINOV, A., COLE, D.B., BEHL, R.J., PLANAVSKY, N.J., LYONS, T.W. (2022) Variable local basin hydrography and productivity control the uranium isotope paleoredox proxy in anoxic black shales. *Geochimica et Cosmochimica Acta*. 317, 433–456. <https://doi.org/10.1016/j.gca.2021.10.011>
- MOLINAS, M., FAIZOVA, R., BROWN, A., GALANZEW, J., SCHACHERL, B., BARTOVA, B., MEIBOM, K.L., VITOVA, T., MAZZANTI, M. and BERNIER-LATMANI, R. (2021) Biological reduction of a U(V)-organic ligand complex. *Environmental Science and Technology* 55, 4753–4761. <https://doi.org/10.1021/acs.est.0c06633>
- MOLINAS, M., MEIBOM, K.L., FAIZOVA, R., MAZZANTI, M., BERNIER-LATMANI, R. (2023) Mechanism of reduction of aqueous U(V)-dpaea and solid-phase U(VI)-dpaea complexes: The role of multiheme *c*-type cytochromes. *Environmental Science and Technology* 57, 7537–7546. <https://doi.org/10.1021/acs.est.3c00666>
- MONTROYA-PINO, C., ANBAR, A.D., VAN DE SCHOOTBRUGGE, B., OSCHMANN, W., PROSS, J., ARZ, H.W., WEYER, S. (2010) Global enhancement of ocean anoxia during Oceanic Anoxic Event 2: A quantitative approach using U isotopes. *Geology* 38, 315–318. <https://doi.org/10.1130/G30652.1>
- PAN, Z., BÄRTOVÁ, B., LAGRANGE, T., BUTORIN, S.M., HYATT, N.C., STENNETT, M.C., KVASHNINA, K.O., BERNIER-LATMANI, R. (2020) Nanoscale mechanism of UO_2 formation through uranium reduction by magnetite. *Nature Communications* 11, 1–12. <https://doi.org/10.1038/s41467-020-17795-0>
- ROBERTS, H.E., MORRIS, K., LAW, G.T.W., MOSSELMANS, J.F.W., BOTS, P., KVASHNINA, K., SHAW, S. (2017) Uranium(V) incorporation mechanisms and stability in Fe(II)/Fe(III) (oxyhydr)oxides. *Environmental Science and Technology Letters* 4, 421–426. <https://doi.org/10.1021/acs.estlett.7b00348>
- SATO, A., BERNIER-LATMANI, R., HADA, M., ABE, M. (2021) *Ab initio* and steady-state models for uranium isotope fractionation in multi-step biotic and abiotic reduction. *Geochimica et Cosmochimica Acta* 307, 212–227. <https://doi.org/10.1016/j.gca.2021.05.044>
- SCHAUBLE, E.A. (2007) Role of nuclear volume in driving equilibrium stable isotope fractionation of mercury, thallium, and other very heavy elements. *Geochimica et Cosmochimica Acta* 71, 2170–2189. <https://doi.org/10.1016/j.gca.2007.02.004>
- STIRLING, C.H., ANDERSEN, M.B., WARTHMAN, R., HALLIDAY, A.N. (2015) Isotope fractionation of ^{238}U and ^{235}U during biologically-mediated uranium reduction. *Geochimica et Cosmochimica Acta* 163, 200–218. <https://doi.org/10.1016/j.gca.2015.03.017>
- STYLO, M., NEUBERT, N., WANG, Y., MONGA, N., ROMANIELLO, S.J., WEYER, S., BERNIER-LATMANI, R. (2015) Uranium isotopes fingerprint biotic reduction. *Proceedings of the National Academy of Sciences of the United States of America* 112, 5619–24. <https://doi.org/10.1073/pnas.1421841112>
- VETTESE, G.F., MORRIS, K., NATRAJAN, L.S., SHAW, S., VITOVA, T., GALANZEW, J., JONES, D.L. and LLOYD, J.R. (2020) Multiple lines of evidence identify U(V) as a key intermediate during U(VI) reduction by *Shewanella oneidensis* MR1. *Environmental Science and Technology* 54, 2268–2276. <https://doi.org/10.1021/acs.est.9b05285>
- WANG, X., JOHNSON, T.M., LUNDSTROM, C.C. (2015) Low temperature equilibrium isotope fractionation and isotope exchange kinetics between U(IV) and U(VI). *Geochimica et Cosmochimica Acta* 158, 262–275. <https://doi.org/10.1016/j.gca.2015.03.006>
- WEYER, S., ANBAR, A.D., GERDES, A., GORDON, G.W., ALGEO, T.J., BOYLE, E.A. (2008) Natural fractionation of $^{238}\text{U}/^{235}\text{U}$. *Geochimica et Cosmochimica Acta* 72, 345–359. <https://doi.org/10.1016/j.gca.2007.11.012>

