# Non-crystalline tetravalent uranium species in the subsurface: formation and stability

Thèse N° 8952

Présentée le 5 avril 2019

à la Faculté de l'environnement naturel, architectural et construit Laboratoire de microbiologie environnementale Programme doctoral en génie civil et environnement

pour l'obtention du grade de Docteur ès Sciences

par

### Luca LOREGGIAN

Acceptée sur proposition du jury

Prof. A. Buttler, président du jury Prof. R. Bernier-Latmani, directrice de thèse Prof. J. Peña, rapporteuse Prof. S. Shaw, rapporteur Prof. A. Meibom, rapporteur



## Acknowledgments

My work at EPFL was challenging and tough. Luckily, I was surrounded by people that always encouraged and supported me.

First and foremost, I would like to express my sincere gratitude to Rizlan Bernier-Latmani for giving me the opportunity to work on this project and for the experiences that I gained with it. I am extremely grateful for her constant support, for her guidance, advice and (most of all) patience through this process. She patiently followed my shaky steps, she addressed me to the right path pushing me to do better and go a little bit further. She has been a tremendous source of inspiration. Her expertise, curiosity and tireless dedication for new discoveries are simply unique.

I would like to thank Ashley, Zezhen, Gabrielle and Margaux; this thesis would not have been the same without their help, scientific advice and the time that they have kindly shared for discussions. I am also extremely gratefull to present and former collegues from EML. EML is fantastic group of people who cheered me up in the moments of discouragements. Special thanks go also to Gosja, Matt, Karim, Mapi, Conny, Solenne, Karen, Emma, Aislinn, Niels, Barbora, Maria and Manon. Moreover, I am grateful to Karin and Mapi who shared the office with me, they did a great job bearing my daily mess and my unstable mood during my whole time at EML. I would also like to express my gratitude to Prof. Laura Wasilenky. I really appreciated the enlightening discussions that we had during her stay in Lausanne.

It was a great pleasure to to be accompanied and to meet supportive people during my time at synchrotrons. Beamtime shifts were made bearable by the many people I shared them with. These persons made my exisestence at the beamline a bit easier or at least more pleasent. Although I learned most of the XAS

ш

fundamentals on books or the hard way through my own mistakes, in a few occasions I was luckily helped by Yuheng who patiently teached me a few tricks that I would have never learned on books. Special thanks go also to Thomas, Mapi, Zezhen, and Pierre. Finally, I would like to thank Vincent and Pierre that were always available to share a little bit of free time in between samples changes or a cup of coffee during the neverending nights at the beamline. Pierre and Vincent are for me an example of dedication and competence that I also wish to achieve in my professional life.

A special shout out go to my flatmates and to all the friends with whom I shared the little free time available. Thanks to their contagious cheerfulness, I was able to forget the troubles for a while and gain new energy for the challenges that I needed to face the day after.

I would like to thank my family; Dad, Mum, Elisa and Killo who always believed I could do it even during the times I actually did not.

Finally, I couldn't have completed this without Sara. She tried her hardest to pull me out from EPFL in multiple occasions. Her smile and care revived my mental health and turned the worst day into a distant memory.

Lausanne, November 7<sup>th</sup> 20

## Abstract

Uranium (U) contamination of ground and surface waters poses an acute hazard on the ecosystem and human health. Since the discovery of microbial U(VI) reduction, U bioremediation has been explored as a promising and cost-effective method compared to traditional treatments. The speciation of the bioreduction product was originally stated to be uraninite (UO<sub>2</sub>) which is a recalcitrance crystalline U(IV) species. On the other hand, recent studies demonstrated that non-crystalline species (NCU4) are the dominant product of bioreduction. Since NCU4 species are more labile than UO<sub>2</sub>, new concerns are associated with the long-term stability of these U(IV) products. In fact, the effectiveness of bioremediation depends on the resistance of NCU4 species to oxidation and remobilization into solution.

In this regard, it was previously hypothesized that aging might transform NCU4 to UO<sub>2</sub> that would enhance the resistance of U(IV) to oxidation and release into the aqueous phase. We investigated this hypothesis by incubating NCU4 species immobilized in natural sediments under anoxic conditions for a period of 12 months. We systematically probe the speciation of U in the sediment using X-ray absorption spectroscopy. Under the investigated conditions, NCU4 does not age to UO<sub>2</sub>. Thus, it is likely that NCU4 produced during bioremediation persists in the subsurface even for an extended period of time if anoxia is maintained. Therefore, the remediated site remains vulnerable to events that bring oxygen into the reduced zone.

In fact, NCU4 species are rapidly oxidized upon exposure to oxygen. Furthermore, we demonstrated that under certain conditions (i.e., high dissolved oxygen (DO) concentration), the presence of FeS accelerates the oxidation and re-mobilization of NCU4 via the production of reactive oxygen species. Since ROS production during FeS oxidation depends on the concentration of DO and the speciation of Fe(II) in the sediments, at low DO concentrations, low amounts of ROS were detected, and the enhancement of U(IV) oxidation by FeS became negligible.

Moreover, this thesis investigates the isotopic fractionation ( $^{238}U/^{235}U$ ) during abiotic reduction by magnetite (Fe<sub>3</sub>O<sub>4</sub>), a Fe(II) bearing mineral capable of reducing U(VI) that is commonly found in bioreduced zones. The results of preliminary experiments confirmed that U reduction by Fe<sub>3</sub>O<sub>4</sub> has opposite fractionation than predicted by the nuclear field shift effect suggesting that a kinetic effect may drive fractionation during reduction. Preliminary XAS speciation of U immobilized on the surface of magnetite report that U(V) might occurs during reduction.

Keywords: Uranium, bioremediation. aging, stability, isotopes fractionation

## Riassunto

La contaminazione da uranio (U) del terreno e delle acque superficiali rappresenta un rischio severo per l'ecosistema e la salute umana. Dalla scoperta della riduzione biotica di U(VI), il biorisanamento da U è stato esplorato come metodo promettente ed economico rispetto ai trattamenti piu tradizionali. D'altra parte, originariamente l'uraninite (UO<sub>2</sub>), una specie U(IV) cristallina recalcitrante, era ritenuta l'unico prodotto del biorisanamento. Studi recenti hanno dimostrato che le specie non cristalline (NCU4) sono il prodotto dominante della bioriduzione. Poiché le specie NCU4 sono più labili di UO<sub>2</sub>, la stabilità nel lungo termine di questi prodotti U (IV) rappresentano una criticità per l'applicazione di questa tecnica. Infatti, l'efficacia del biorisanamento dipende dalla resistenza di NCU4 all'ossidazione e alla sua rimobilitazione in soluzione. A questo proposito, è stato precedentemente ipotizzato che l'invecchiamento possa trasformare NCU4 in UO<sub>2</sub> e migliorare la sua resistenza all'ossidazione. Abbiamo investigato questa ipotesi incubando le specie NCU4 immobilizzate in sedimenti naturali in condizioni anossiche per un periodo di 12 mesi. L' analisi sistematica della speciazione di U immobilizzata nel sedimento attraverso la spettroscopia a raggi X (XAS) ha rivelato che, alle condizioni investigate, NCU4 non si trasforma in UO<sub>2</sub>. Quindi è probabile che NCU4, prodotto durante il biorisanamento, persista nel sottosuolo se mantenuta in condizioni anossiche. Pertanto il sito rimediato rimane vulnerabile agli eventi che portano ossigeno nella zona biostimolata.

In effetti, le specie NCU4 sono rapidamente ossidate in seguito all'esposizione all'ossigeno. Inoltre, riconosciamo che in determinate condizioni (cioè concentrazione di ossigeno disciolto (DO) elevato), la presenza di mackinawite (FeS) accelera l'ossidazione e la mobilizzazione di NCU4 attraverso la produzione di specie radicali liberi dell'ossigeno (ROS). Abbiamo osservato che la produzione di ROS durante l'ossidazione di FeS dipende dalla concentrazione di DO e dalla speciazione dei minerali ferrosi contenuti nei sedimenti. A basse concentrazioni di DO, sono state rilevate basse quantità di ROS e la rapida ossidazione di U(IV) da parte di FeS è divenuta trascurabile. Inoltre, questa tesi indaga il frazionamento isotopico (238U / 235U) durante la riduzione abiotica mediata da magnetite (Fe3O4), un ossido di ferro capace di ridurre U(VI) che si trova comunemente nelle zone bioredotte. Il frazionamento che abbiamo osservato indica che la magnetite reduce prevalentmente l'isotopo pesante. Inoltre la speciazione preliminare mediante XAS suggerisce che U(V) è presente e stabile sulla superficie della magnetite.

## Contents

Acknowled	gmentsI	11
Abstract		v
Riassunto	V	11
Contents		Х
List of figur	res X	v
List of table	esXI	Х
Chapter 1	Introduction	1
1.1	Uranium Bioremediation	3
1.2	Aging of non-crystalline tetravalent uranium	4
1.3	Stability of NCU4 and the impact of mackinawite	6
1.4	Uranium isotopic fractionation	9
1.5	Objectives of the thesis1	1
	1.5.1 The effect of aging on the speciation of non-crystalline U(IV)1	2
	1.5.2 The role of iron sulfides in the stability of non-crystalline U(IV)1	3

	1.5.3 Uranium isotope fractionation during reduction mediated by magnetite
1.6	References16
Chapter 2	The effect of aging on the structure of non-crystalline tetravalent uranium25
2.1	Abstract
2.2	Introduction27
2.3	Materials and methods29
	2.3.1 Description of the sediments
	2.3.2 Design and operation of the column experiments
	2.3.3 Characterization of the effluent composition
	2.3.4 Chemistry of the sediments
	2.3.5 X-ray absorption spectroscopy (XAS)
	2.3.6 XAS Data Analysis
	2.3.7 Chemical extraction
	2.3.8 Incubation of SRC sediments for the aging of bioreduced species of U(IV)
2.4	Results and discussion35
	2.4.1 Bioreduction of U(VI) under sulfate-reducing conditions
	2.4.2 Chemical characterization of bioreduced sediments
	2.4.3 Uranium speciation in the initial and aged samples
2.5	Environmental Implications42
2.6	Acknowledgments43

2.7	References
Chapter 3	The role of iron sulfide phases in the stability of non-crystalline tetravalent uranium in
natu	ral sediments49
3.1	Abstract50
3.2	Introduction51
3.3	Materials and methods52
	3.3.1 Sediments
	3.3.2 Batch oxidation experiments
	3.3.3 Flow-through oxidation experiments54
	3.3.4 Analyses55
	3.3.5 Hydrogen peroxide analysis
3.4	Results and Discussion57
	3.4.1 U and Fe speciation in the sediments57
	3.4.2 Hydrogen peroxide production
	3.4.3 Uranium oxidation in batch experiments59
	3.4.4 Uranium oxidation in flow-through experiments
3.5	Environmental implications68
3.6	Acknowledgments
3.7	References70
Chapter 4	Uranium isotope fractionation during reduction of U(VI) by magnetite75

4.1	Abstract76
4.2	Introduction77
4.3	Materials and Methods79
	4.3.1 Synthesis of magnetite and maghemite79
	4.3.2 Characterization of magnetite79
	4.3.3 Uranium adsorption and reduction experiments80
	4.3.4 MC ICP-MS uranium analysis81
	4.3.5 X-ray absorption spectroscopy measurements82
	4.3.6 XAS Data Analysis83
4.4	Results and discussion83
	4.4.1 Uranium reduction and isotope fractionation83
	4.4.2 Average valence state of uranium87
4.5	Acknowledgments93
4.6	References94
Chapter 5	Conclusions97
5.1	Outlook
Annex 1 S	upporting information Chpt. 2109
Spe	ciation of sulfur110
Sca	nning transmission electron microscocopy110
Annex 2 S	upporting information Chpt. 3

Curri	culum vitae	165
Anne	x 3 Supporting information Chpt. 4	151
	Kinetic modeling	133
	The effect of bicarbonate concentration in RAGW	132
	Characterization of IRC sediment	132

## List of figures

Figure 3.2 Uranium speciation over time during the batch oxidation experiment under oxic conditions for (a) SRC sediments and (b) IRC sediments. Solid lines represent the

experiment with no amendment, and dotted lines represent the amendment of SOD
and CAT61
Figure 3.3 S K-edge XANES speciation in SRC sediments before and after oxidation
under oxic conditions in (a) batch experiments and (b) flow-through experiments. Solid
lines represent the data while dashed lines represent the models63
Figure 3.4 Uranium speciation over time during the flow-though oxidation experiment
at 10 mM HCO $_3$ under (a) oxic and (b) suboxic conditions in SRC sediments (solid lines)
and IRC sediments (dashed lines)65
Figure 3.5 Fe K Edge EXAFS speciation before and after oxidation in CSTR experiment
for SRC (a) and IRC (b) sediments67
Figure 4.1 Concentration of U(VI) over time in U(VI) reduction experiments with
magnetite as a reductant with 200 $\mu M$ U initial concentration (a) and 80 $\mu M$ U initial
concentration (c) in duplicates. $\delta$ 238U values of remaining U(VI) (solid phase extracted)
plotted against the fraction of U(VI) reduced (C/C0) at 200 $\mu M$ U initial concentration
(b) and 80 $\mu M$ U initial concentration (d) in duplicates
Figure 4.2 140 $\mu M$ U initial concentration (c). $\delta^{238} U$ values plotted against the fraction
of U(VI) adsorbed in experiments (C/C_) with 80 $\mu M$ U and 140 $\mu M$ U initial
concentration (d)86
Figure 4.3 Normalized bulk U $L_{\mbox{\tiny III}}$ -edge XANES spectra of selected samples reported with
the correspondent U:Fe molar in the experiment (black line), their LCFs (red dashed
line) using U(VI) adsorbed on ferrihydrite as standard for U(VI) (blue), biogenic UO $_2$ as
standard for U(IV) (yellow) and $U_3O_8$ as standard for U(V) and U(VI) (dark red). Fit
results are reported in

### List of tables

Table 2.1 Results of shell-by-shell fitting procedure of U LIII edge EXAFS spectra of SRC 2 after bioreduction phase (no aging) and after an aging period of 4, 8 and 12 months Table 3.1 LCF of the Fe K-edge EXAFS data for RABS as well as in SRC and IRC sediments before oxidation. (-) Reference compound not required for the fit. The data for RABS and SRC were already presented in Chapter 2......58 Table 3.2 results of LCF analysis of S K-edge XANES spectra of SRC sediments before and after oxidation in batch experiments under oxic conditions. ......63 Table 3.3 Fe K-edge EXAFS speciation in SRC and IRC sediments before and after oxidation under oxic flow-through experiments. The data for RABS and the initial Table 4.1 Results of bulk U L<sub>III</sub> edge XANES linear combination fit of U(VI) reduction in batches with Fe<sub>3</sub>O<sub>4</sub> or PO4 sorbed magnetite at various U concentration and U(VI) adsorption on  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>. (-) standard not required for the fit based on the X<sub>r</sub><sup>2</sup> (Table SI-1). Values in parenthesis are the uncertainties of the LCF from Athena<sup>16</sup>. The content of U(VI) and U(VI) is corrected based on the LCF in Table SI-3 as U3O8 reference compound consist of 2 U(V) and 1 U(VI) ......89

Table 4.2 U(VI) reduction in separate batches containing 5mM Fe as Fe3O4 and 80 $\mu M$
U; concentration of dissolved U over time, Isotopic fractionation of U ( $\delta^{^{238}}$ U) remaining
in the aqueous phase in per mil (‰) and results of the linear combination fit (LCF)
analysis by bulk U LIII-edge XANES in percentages (%). The values in parenthesis are
the uncertainties of the LCF from Athena <sup>16</sup> . The content of U(VI) and U(VI) is corrected
based on the LCF in Table SI-4 as ${\sf U}_3{\sf O}_8$ reference compound consist of 2 U(V) and 1
U(VI)

# Chapter 1 Introduction

This opening chapter provides the context of the work discussed in the thesis. It introduces the principles of in-situ uranium bioremediation as a remediation strategy and the concerns associated with this technique. The effect of aging on the speciation of U(IV) and the stability of non-crystalline tetravalent uranium are also discussed. Furthermore, the isotopic fractionation of U and its potential as a tool to monitor uranium reduction are discussed. In the end, the objectives of each of the following chapters are presented.

Uranium (U) contamination of surface and groundwater is the legacy of decades of U mining and processing, nuclear power generation, and weapons production. Contaminated areas are globally ubiquitous, and their remediation is required when the concentration is sufficiently high to pose a threat to human health.

The mobility of U in the subsurface depends on pH, Eh, U oxidation state and its speciation<sup>1</sup>. Under oxidizing conditions, U is generally present in the hexavalent oxidation state (U(VI)). U(VI) is found as uranyl cations or in hydroxyl complexes below pH 6.5. At higher pH, the solubility of U(VI) is enhanced by complexation with carbonate in uranyl carbonato complexes (i.e., carbonato, dicarbonato, and tricarbonato species depending on the pH). At high pH and low dissolved inorganic carbon (DIC), uranium is likely to be adsorbed on the surface of iron oxides.<sup>2</sup> Conversely, tetravalent U (U(IV)) is sparingly soluble and less mobile even in the presence of ligands such as carbonate. For this reason, *in-situ* reduction of U(VI) to U(IV) is proposed as a potential remediation strategy<sup>2</sup>. Since the discovery of microbial U(VI) reduction in 1991<sup>3</sup>, U bioremediation has been explored as a promising and cost-effective method compared to traditional and more expensive treatment.

The speciation of bioreduced uranium was initially believed to be the crystalline and recalcitrant uraninite  $(UO_2)$ , but biogenic  $UO_2$  nanoparticles (bio $UO_2$ )<sup>4</sup> and non-crystalline U(IV) species (NCU4)<sup>5</sup> have been discovered in the last decade. More recently, numerous studies report that NCU4 is the dominant product of biotic reduction under environmental conditions<sup>6–8</sup>. Consequentially, as NCU4 is more labile than crystalline  $UO_2^9$ , the use of bioremediation is now under debate. Although numerous studies demonstrated that microbial U(VI) reduction effectively removes U(VI) from groundwater in the short term, significant concerns stem from whether NCU4 species are stable in the long term.

#### 1.1 Uranium Bioremediation

Since the discovery of U(VI) bioreduction<sup>10</sup>, *in-situ* uranium bioremediation has been investigated as an alternative technique to traditional and more expensive strategies. In fact, while bioremediation relies solely on organic electron donors, for example relatively cheap sources of organic carbon such as ethanol or acetate, and harnesses the power of indigenous microorganisms, physicochemical methods require the use of an engineered reductant and involve a means of delivery to the subsurface. Furthermore, more traditional methods, such as excavation, also require the treatment of the excavated soil, creating a hazard for human health.

Bioremediation of U contaminated groundwater typically involves the injection of electron donors into the subsurface in order to stimulate microbial processes within the plume of the contaminant. After depletion of the most favorable electron acceptors (i.e., O<sub>2</sub>, NO<sub>3</sub>, and Mn(IV)), iron- and sulfate-reducing conditions are typically established simultaneously if sufficient electron donor is available. Under these conditions, many dissimilatory metal- (DMRB) and sulfate-reducing bacteria (SRB) are capable of the enzymatic reduction of U(VI) to U(IV)<sup>2</sup>. In fact, U(VI) has a similar redox couple as Fe(III). Thus, Fe(III)-reducing bacteria are able to reduceU(VI) via the same enzymes that drive the electron transfer to soluble and insoluble Fe(III) species<sup>10</sup>. In addition, biogenic Fe(II)-bearing minerals (i.e., mackinawite and magnetite), as by-products of microbial metabolism, also contribute to U(VI) reduction and immobilization of U(IV) into the sediments<sup>11,12</sup>. The effectiveness of bioremediation has been successfully demonstrated in numerous *in-situ* investigations across a range of environments that includes alluvial sediments, fractured saprolite, and ISR mining sites <sup>13</sup>.

Crystalline uraninite (UO<sub>2</sub>), the most desired U(IV) product due to its recalcitrance to oxidation, was long assumed to be the sole product of bioremediation. However, electron microscopy and X-ray absorption

spectroscopy (XAS) studies have demonstrated that poorly ordered  $UO_2$  nanoparticles and non-crystalline U(IV) are more likely to form<sup>5,14</sup>.

Biogenic uraninite nanoparticles (bioUO<sub>2</sub>) are found during U(VI) bioreduction in pure cultures of SRB and DMRB under specific conditions<sup>4,15–17</sup>. UO<sub>2+x</sub> nanoparticles are found with x values as high as 0.25<sup>4</sup>. Despite the fact that bioUO<sub>2</sub> may form in microenvironments with specific conditions<sup>15</sup>, NCU4 is the main product of enzymatic U(VI) reduction in the natural environment<sup>18–21</sup> and in laboratory studies under environment tally-relevant conditions<sup>15,22</sup>. Indeed, biogenic UO<sub>2</sub> is rarely observed in bioremediation scenarios<sup>23</sup>. NCU4 species include U(IV) associated with biomass, precipitates of poorly ordered solids complexed, for examples with carbonate, and phosphate ligands<sup>7,21,24,25</sup> or adsorbed on oxides<sup>26</sup>. Other than direct enzymatic reduction, NCU4 can also form as a product of U(VI) reduction by Fe(II)-bearing minerals such as biogenic vivianite or magnetite that is presorbed with phosphate<sup>11</sup>.

Recent studies have shown that NCU4 species are more reactive than UO<sub>2</sub> and are readily oxidized and released into the aqueous phase<sup>9,27</sup>. Indeed, the new concern associated with bioremediation is the long-term stability of the U(IV) products following the cessation of remediation treatments. In fact, seasonal watershed dynamics in the aquifer and variation of dissolved oxygen concentration may result in the exposure of bioremediated sites to oxygen. Therefore, the resistance of U(IV) species to oxidation and remobilization into solution is a critical factor for the success of the treatment.

#### 1.2 Aging of non-crystalline tetravalent uranium

Aging of amorphous species has been hypothesized to transform NCU4 to  $UO_2$  and potentially enhance the stability of U(IV) products immobilized in the sediments. For example, the partial transformation of NCU4 to crystalline  $UO_2$  after aging was reported in sediments from Oak Ridge, TN,<sup>28</sup> and Sellafield, UK,<sup>29</sup> after incubation in batch experiments for 11 and 15 months, respectively. Bulk XAS analysis was applied

#### **Chapter 1 Introduction**

to investigate the average speciation of U in the solids over time. The increasing content of crystalline U(IV) species in the sediments was evidenced by the increasing number of U atoms in U-U path of uraninite (i.e., the coordination number, CN) at 3.8Å of the shell-by-shell fit model of the EXAFS region. This translates visually into the growing amplitude of the peak at 3.7 Å in the Fourier transform of the EXAFS.

Kelly et *al.*<sup>28</sup> mimicked *in situ* bioreduction in a batch incubation with natural sediments from Oak Ridge, TN, in artificial groundwater. The U valence state was monitored by XANES linear combination fitting and indicated that reduction reached a maximum of 80% after 5 months and did not proceed further in the next 6 months. Whilst the coordination environment of U was assessed by EXAFS at the beginning and end of the experiment; no intermediate time points were available to monitor progressive changes of speciation through time. Shell-by-shell fitting indicated that a significant amount of UO<sub>2</sub> was present at 11 months (i.e., a CN of 5.5 for the U-U path, typical of bioUO<sub>2</sub>), but the content of UO<sub>2</sub> at the end of the reduction phase (i.e., after 5 months, but before significant aging) was not known, making it difficult to ascertain whether NCU4 aged to form UO<sub>2</sub> or whether UO<sub>2</sub> was present at the onset of the aging experiment. Therefore, there was no conclusive evidence for the transformation of NCU4 to UO<sub>2</sub>.

Newsome et *al.* <sup>28</sup> performed a similar experiment using sediments from Sellafield, UK, that were biostimulated for the reduction of U. This study provides the initial speciation of U prior to aging and demonstrate that U was 100% amorphous U(IV) complexed with bidentate carbon and monodentate phosphorus at the beginning of the aging period. Hence, the significant growth in the amplitude of the peak at 3.8 Å in the Fourier transform (i.e., indicative of the U-U bonding) after 15 months of incubation is interpreted as evidence for the transformation of amorphous U(IV) to UO<sub>2</sub>. Based on the CN of U atoms at 3.8 Å, it was possible to estimate that 20-30% of UO<sub>2</sub> was formed during aging. Despite the transformation of NCU4 to UO<sub>2</sub>, U was rapidly re-mobilized during oxidation experiment at a similar rate as in non-aged samples. This surprising result was interpreted through the formation of nanoparticulate UO<sub>2</sub> with similar reactivity as NCU4 <sup>9</sup> but remains difficult to explain.

On the other hand, a few studies report that NCU4 species persist in the environment and do not transform to UO<sub>2</sub><sup>8,19,30</sup>. Bargar et al.<sup>19</sup> characterized U(IV) in bioreduced sediments after a period of 12 months of aging in column experiments that were deployed *in situ* in the U contaminated aquifer of Rifle, CO (a former vanadium and uranium mining site). Whilst XAS analysis of aged samples showed that the coordination number of the U-U pair correlation doubled with respect to the non-aged sample, this increase was attributed to the partial loss of NCU4 during a bicarbonate flush step prior to sampling for solid speciation. Therefore, when accounting for this loss, the relative content of UO<sub>2</sub> to the total uranium in the system remained the same. Thus, the authors concluded that the initial content of UO<sub>2</sub> did not change after one year of aging in the field, but the occurrence of two distinct phases was interpreted as the end-products of two distinctive reductive pathways; NCU4 from enzymatic reduction and UO<sub>2</sub> from the abiotic reduction mediated by Fe(II)-bearing minerals. Furthermore, NCU4 has also identified in roll front deposit Wyoming, USA, <sup>30</sup> where uraninite initially was believed to be the dominant species<sup>31</sup>. Finally, NCU4 species have also been reported in decades-old U-contaminated wetlands in Les Sagnes<sup>8</sup>, and in Brittany<sup>25</sup>, France. Again, this provides further evidence that NCU4 species may, in fact, be persistent in the environment under anoxic conditions.

In conclusion, although NCU4 is found to be persistent in certain environments, there remain questions about its ability to transform into  $UO_2$  as demonstrated by Newsome et al.<sup>32</sup>.

### 1.3 Stability of NCU4 and the impact of mackinawite

Seasonal watershed dynamics in the aquifer or fortuitous rainfall events may lead to the increase of the dissolved oxygen concentration in groundwater which may enter into bioreduced zones and challenge the stability of U(IV). In this scenario, the recalcitrance of U(IV) species to oxidation will regulate the

6

remobilization of U into solution. Although U(IV) is potentially oxidized by several oxidants such as oxygen, intermediates of denitrification (nitrite, nitrous oxide, and nitric oxide)<sup>33</sup>, Mn(IV) (hydr)oxide and Fe(III) (hydr)oxides, much work has focused on molecular oxygen as exposure to it represents the extreme scenario when assessing re-oxidation.

The oxidation of U by oxygen and release into solution occurs in a three-step mechanism: oxidation of U(IV) to U(VI) by transfer of electrons to oxygen, coordination of a ligand with U(VI), and formation of a soluble U(VI)-ligand complex <sup>27</sup>. Therefore, several factors impact the oxidation and remobilization of U(IV) at circumneutral pH: the concentration of oxidants, the concentration of ligands (with carbonate being the most important in most circumneutral groundwaters) and the reactivity of the U(IV) species.

The presence of carbonate promotes the dissolution of U, facilitating the detachment of surfaceassociated U(VI) under oxidizing conditions. Hence, it prevents the passivation of the active surface which would slow down the reaction<sup>9,34</sup>. Oxidation of U(IV) species and U release has been studied in the laboratory<sup>9,34</sup>; these findings indicate that NCU4 is slightly more susceptible to oxidation than bioUO<sub>2</sub> nanoparticles in the presence of dissolved oxygen. Indeed, the structure and the active surface differ between the two U(IV) species: while NCU4 species are amorphous, UO<sub>2</sub> is highly structured with limited U atoms having surface coordination. BioUO<sub>2</sub> nanoparticles have the same structure as UO<sub>2</sub>, but their nano-size enhances their reactivity since a higher percentage of the U atoms is found at the oxide-water interface<sup>23</sup>.

In the subsurface, bioreduced species of U(IV) are found in association with other reduced species such as Fe(II)-bearing minerals or iron sulfides which are by-products of the anaerobic metabolism of sulfatereducing and iron-reducing bacteria. These species have been proposed to enhance the effectiveness of bioremediation by buffering the oxidation-reduction potential of groundwater and hence indirectly protecting U(IV) from oxidation<sup>23,35</sup>. This protective mechanism was first invoked from a laboratory column experiment in which natural sediments containing bioreduced U(IV) species and mackinawite (FeS) were exposed to oxic groundwater<sup>36</sup>. Further investigations of column<sup>37</sup> and flow-through experiments<sup>38,39</sup> confirmed that FeS is capable of temporarily protecting UO<sub>2</sub> from oxidation and dissolution. In these experiments, an initial transition stage was observed during which no U(VI) was mobilized<sup>38,39</sup>. However, it should be noted that UO<sub>2</sub> is only a minor U(IV) species in the subsurface and it is rarely found under environmental conditions after U(VI) bioreduction.

On the other hand, when the protective effect of FeS was tested against the oxidation of NCU4 by O<sub>2</sub>, in a similar experimental set-up <sup>38</sup>, U was rapidly oxidized and released into the aqueous phase after the addition of FeS<sup>40</sup>. The authors proposed that FeS indirectly accelerates U mobilization through the formation of a transient reactive species of Fe(III) that oxidizes U(IV). This mechanism relies on a direct reaction between solid Fe(III) and NCU4 to allow electron transfer. However, despite the apparent co-location of FeS and NCU4 at the sediment grain size scale, micro-XAS/XRF<sup>41</sup> and EDS coupled to scanning electron microscopy<sup>6</sup> have revealed that uranium and Fe are not necessarily in direct proximity at the micron scale. Hence, solid-solid electron transfer between Fe(III) and NCU4 may be limited in the environment, where contact between NCU4 and FeS is perhaps uncommon. Whilst being possibly valid in a laboratory system as that of Bi et al.<sup>40</sup> where synthetic FeS and NCU4 were homogeneously mixed in continuously stirred reactors, this model may not apply to the environment where direct contact between the two solid species is limited.

Similar oxidative transformation induced by oxidation of FeS was reported for As(III). In this case, the oxidation of As(III) was attributed to the production of reactive oxygen species (ROS) generated by the oxidation of structural Fe(II) in FeS<sup>42,43</sup>. ROS such as superoxide, hydrogen peroxide and hydroxyl radicals are intermediate products of the sequential one-electron step reduction of oxygen to water. Previously, ROS were assumed to be generated via photochemical processes, but recent *in-situ* investigations<sup>44,45</sup> and laboratory studies<sup>46–48</sup> provide evidence for the light-independent generation of ROS. Due to their high

reactivity, ROS are transient species in nature because they are strong oxidants capable of oxidizing numerous redox-sensitive elements, for example, arsenic<sup>48</sup>, iron<sup>49</sup>, copper<sup>50</sup>, and manganese<sup>51</sup>. Murphy et *al.*<sup>46</sup> suggest that, in the subsurface, at the interface between oxic and anoxic zones, the cycling of Fe between ferrous and ferric oxidation states is an essential catalytic mechanism for the production of ROS. Additionally, laboratory experiments have demonstrated the abundant generation of HO<sup>-</sup>, with H<sub>2</sub>O<sub>2</sub> as an intermediate, upon the exposure of reduced sediments containing Fe(II) minerals (i.e., phyllosilicates, and iron-sulfide minerals) to oxygen. Moreover, the efficiency of FeS for the production of HO<sup>-</sup> was higher than for other forms of Fe(II) minerals (i.e., siderite, pyrite and zero valent iron) tested under similar conditions<sup>47</sup>. The proposed mechanism of ROS production via the oxygenation of Fe(II) species consists of a cascade reaction as elucidated by Murphy et *al.*<sup>46</sup>; the oxidation of Fe(II) species first yield a superoxide anion radical (O<sub>2</sub><sup>•-</sup>), which can react with Fe(II) or disproportionate to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). In turn, H<sub>2</sub>O<sub>2</sub> can react with transition metals and yields a hydroxyl radical (HO<sup>-</sup>), which is the most reactive ROS, and it is capable of oxidizing most organic contaminants and redox-sensitive elements.

#### 1.4 Uranium isotopic fractionation

Natural U has five isotopes with <sup>238</sup>U and <sup>235</sup>U being the primordial ones. They respectively represent 99.28% and 0.72% of natural uranium which are sufficiently high concentration to be measured by multicollector-inductively coupled plasma-mass spectrometers (MC-ICP-MS). A minute fraction of <sup>236</sup>U can also naturally occur (i.e., <sup>236</sup>U/<sup>238</sup>U <10<sup>-9</sup>)<sup>52</sup>. <sup>238</sup>U and <sup>235</sup>U are two parent isotopes of the <sup>238</sup>U- and <sup>235</sup>U- series chains and they have half-lives of 4.468\*10<sup>9</sup> years and 0.7038\*10<sup>9</sup> years. Therefore, because these two isotopes have a different decay rate, the absolute <sup>238</sup>U/<sup>235</sup>U has changed from 3.3 to 137.88<sup>52,53</sup> over the 4.6\*10<sup>9</sup> years after the genesis of the solar system. Although U isotopes are not stable isotopes, given the long half-lives of <sup>238</sup>U and <sup>235</sup>U, the natural variations of <sup>238</sup>U/<sup>235</sup>U that are observed on Earth today are independent of radioactive decay processes, and they are generated by chemical reactions that are

capable of fractionation<sup>53</sup>. Significant fractionation effects are expressed in association with a variety of chemical transformations including adsorption, changes in U speciation, or redox process as biotic and abiotic reduction. Recent experimental studies have investigated <sup>238</sup>U/<sup>235</sup>U fractionation during various biotic and abiotic processes. Experimental studies that do not involve U redox changes have shown limited fractionation effects: (i) U(VI) adsorption onto birnessite (Mn-oxyhydroxide) in an oxic solution reported ~0.2‰ lower in the adsorbed pool of U as expected from mass-dependent fractionation<sup>54</sup>; (ii) at the Old Rifle Field site no <sup>238</sup>U/<sup>235</sup>U change was observed in the groundwaters during adsorption<sup>55</sup>; (iii) finally, no fractionation is observed for U incorporation into calcite or aragonite<sup>56</sup>. On the contrary, U redox exchange has been shown to induce a range of distinctive signatures: (i) when soluble U(IV) is oxidized to U(VI) at low pH (0.1 M HCl), the U(VI) pool result in  $\sim$ 1.1‰ lower than U(IV)<sup>57</sup>; (ii) various reduction experiments using sulfate reducing and dissimilatory metal-reducing bacteria have shown that reduction removes U from oxidized solution and enriches the heavier U isotopes in the product of reaction with a fractionation effect that is consistently in the ~1‰ range<sup>58,59</sup>. Conversely, Stylo et al.<sup>58</sup>, after surveying the isotopic signatures of biotic and abiotic reductive pathways, reported that abiotic reduction by Fe(II)-bearing minerals (magnetite, green rust or aqueous Fe(II)) preferentially reduces <sup>235</sup>U, but no fractionation is observed during reduction by mackinawite and aqueous sulfide. Recently, the role of the initial speciation of aqueous U(VI) has been investigated. This work shows that, when Ca-UO<sub>2</sub>-CO<sub>3</sub> is the primary species of soluble U(VI), U(VI) reduction by mackinawite display an isotopic signature that is comparable to biotic reduction<sup>60</sup>. Overall, these findings highlight the fact that the observed isotope fractionation depends on a multitude of factors such as the mechanism of the reaction, the reaction rate, and the speciation of reactants.

Although the mechanism of fractionation is not fully understood yet, considerable interest has been shown about its potential application to monitor redox processes such as the case of the remediation of U ore mines. In this regard, the attenuation of groundwater contamination is traditionally monitored via measurements of the contaminant concentration in the aquifer, but this method is affected by strong

#### **Chapter 1 Introduction**

uncertainties that are related to concomitant processes such as adsorption or dilution. U isotopes fractionation was measured at the Old Rifle site during an *in-situ* bioremediation trial to test the feasibility of the tool on the field<sup>61</sup>; this work reported that *in-situ* biostimulation of metal-reducing bacteria for the removal of U shows the same isotopic signature as U bioreduction in laboratory experiments<sup>58,59</sup>.

Despite the fact that the <sup>238</sup>U/<sup>235</sup>U ratio has been used as a marker to monitor the reduction in the subsurface, the mechanism of isotopic fractionation is not well characterized, and further investigation is warranted. One question that remains to be investigated is the nature of the factors controlling isotope fractionation during U(VI) reduction by Fe(II)-bearing minerals. Since Fe(II)-bearing minerals are ubiquitous in reduced zones and are efficient reductants, this reductive pathway is a relevant process contributing to the immobilization of U in the environment. Among the others, magnetite is an iron oxide of particular interest because it frequently occurs in reduced environments, for example in naturally reduced zones in the Colorado River Basin<sup>62</sup> or at Rifle<sup>63</sup>.

#### 1.5 Objectives of the thesis

Uranium contamination in the subsurface represents an enormous technical and financial challenge for remediation. Although bioremediation has been proposed as an effective and convenient strategy, its efficacy may be questioned because of the extensive formation of potentially labile non-crystalline U(IV) in the field. This work investigates two aspects that are related to the stability of NCU4 in the environment; (i) the potential transformation of NCU4 to UO<sub>2</sub> under anoxic conditions and (ii) the stability of NCU4 during re-oxidation by O<sub>2</sub>.

For these purposes, we selected sediments from Rifle (CO, USA) to perform columns and batch experiments that replicate the geochemical conditions in the environment.

11

The Old Rifle site is former vanadium and uranium mining and milling processing site situated in the Colorado River Basin, (CO, USA), near the city of Rifle. Uranium was processed on site from 1932 to 1942. The former processing facility contained large piles of mill tailing on site from which residual uranium leached into the unconfined aquifer. Even though the source of contamination was reprocessed and deposited at the New Rifle site (~3 km southwest of the city of Rifle) in 1958, the aquifer still exhibits contamination with low levels of uranium (0.4 to  $1.4 \mu$ M) which are above the maximum contamination limit of  $0.18 \mu$ M. The Old rifle site is now part of the Uranium Mill Tailings Remedial Action (UMTRA) program of the U.S. Department of energy. Over the past 15 years, numerous field-scale experiments were conducted to gain information on the microbial processes and associated geochemistry that control uranium mobility in the subsurface with the intention to assess the potential of bioremediation as a decontamination strategy. Thus, a considerable body of information is available about the geochemical properties of the site, its microbiology, and even the isotopic fractionation of uranium during an *in-situ* bioremediation trial<sup>61</sup>. The in-depth characterization of the site offers the possibility to mimic the environment through laboratory studies.

#### 1.5.1 The effect of aging on the speciation of non-crystalline U(IV)

As previously discussed, EXAFS evidence indicates that a significant amount of UO<sub>2</sub> was formed after a 15 months aging period under anoxic conditions in sediments from Sellafield, UK<sup>29</sup>. If newly formed UO<sub>2</sub> is present as nanoparticles, it is expected that their recalcitrance is not significantly improved if compared to NCU4 species as indicated by Cerrato et *al.*<sup>9</sup>. Hence, if aging transforms NCU4 to UO<sub>2</sub> nanoparticles similar to bioUO<sub>2</sub>, the resistance of aged U(IV) is expected to be similar as in non-aged material.

In contrast, aging of sediment in-situ in a column at Rifle, CO, provided no conclusive evidence of the transformation of NCU4 to UO<sub>2</sub>, indicating that NCU4 might be a persistent species that does not transform to UO<sub>2</sub> in these sediments. However, as the speciation of U is not monitored through time, it is

possible that successive transformations may have occurred during this time due to natural variations of geochemical conditions that occur *in situ*, particularly the DO concentration.

In light of this, the experiments presented in chapter 2 aimed to systematically monitor the speciation of bioreduced species of U(IV) through time and under controlled conditions. The U(IV) species were produced in sediment from Rifle via column-experiments, under flow conditions mimicking the Rifle aquifer. The reduced sediments were incubated in a batch experiment to ensure stable and controlled anoxic conditions through the entire period of aging. Here, XAS was used extensively to systematically assess the speciation of U(IV) in sediments from column-experiments (i.e., prior to aging) versus those aged for 4, 8 and 12 months under stable anoxic conditions.

#### **1.5.2** The role of iron sulfides in the stability of non-crystalline U(IV)

The final speciation of U(IV) after bioremediation depends on various biogeochemical factors, but most studies report that NCU4 is the dominant species found in the environment. Since NCU4 species are more susceptible to oxidation and dissolution than UO<sub>2</sub>, a significant concern exists regarding the long-term sustainability of bioremediation in U contaminated sites. Therefore, understanding the factors affecting U stability is essential to predicting the fate of NCU4 in the environment. In this regard, the role of other reduced metal species which are found in association with U(IV) after biostimulation is poorly understood. Among others, FeS is one of the most abundant by-products of microbial processes during bioremediation in sites where sulfate concentration in groundwater is sufficiently high to sustain sulfate-reducing micro-organisms. Since FeS is very reactive to oxygen, it was initially hypothesized to act as a protecting agent against the oxidation of U(IV). While this hypothesis has been proved for UO<sub>2</sub> <sup>38</sup>, the effect of FeS in the stability of NCU4 is not fully understood yet. In fact, contrary to what is shown for UO<sub>2</sub>, it appears that FeS enhances the oxidation of NCU4 and the release of U(VI) to solution<sup>40</sup>.

The effect of FeS in the stability of NCU4 has been previously investigated only in a laboratory system using synthetic FeS and NCU4 bioreduced in pure bacterial culture. Chapter 3 investigates the role of FeS in a more complex system that better mimics the environment as both NCU4 and FeS are products of a long period of biostimulation of natural sediments in column-experiments. Whilst Bi et al. <sup>40</sup> proposed that NCU4 is oxidized by a reactive Fe(III) species accepting electrons via surface contact with NCU4, this mechanism may only be relevant in a simple system containing only FeS and NCU4 as in the reported experiments<sup>40</sup>. Instead, based on previous work demonstrating dark production of ROS upon the exposure of FeS to O<sub>2</sub><sup>47,48</sup>, we hypothesize that FeS indirectly accelerates the oxidation of NCU4 through the production of ROS. Thus, through a series of batch and flow-through experiments, we investigated whether these soluble and strong oxidants may be involved in the oxidative dissolution of NCU4 in bioreduced sediments from the Rifle site.

#### 1.5.3 Uranium isotope fractionation during reduction mediated by magnetite

Previous work reports that abiotic reduction of U(VI) by Fe(II)-bearing minerals preferentially enriches the light U isotope ( $^{235}$ U) in the products<sup>58</sup>. This isotopic signature is in the opposite direction of what predicted by the nuclear field shift effect and observed during biotic reduction. Stylo et *al.*<sup>58</sup> suggest that a kinetic effect drives the preferential reduction of  $^{235}$ U, but the mechanism of isotopic fractionation during U abiotic reduction by magnetite remains to be uncovered. Furthermore, two alternative reductive pathways were hypothesized: (i) two consecutive one-electron transfers to U(VI) resulting in the formation of U(IV) or (ii) one- electron transfer to U(V) that disproportionates to U(IV) and U(VI)<sup>58</sup>.

The goal of the work presented in chapter 4 is to explore the mechanism of U(VI) reduction by magnetite and the associated isotopic fractionation. For this purpose, we conducted a series of batch experiments using synthetic magnetite (Fe<sub>3</sub>O<sub>4</sub>) as a reductant. To replicate the same conditions as in Stylo et *al.*  $^{58}$ ,  $Fe_3O_4$  was synthesized according to the same protocol. U(VI) reduction by synthetic  $Fe_3O_4$  has been investigated at the following conditions:

(i) Two different U/Fe<sub>3</sub>O<sub>4</sub> loadings to investigate the effect of different reduction rates on the extent of isotopic fractionation; (ii) A set of experiment was conducted with Fe<sub>3</sub>O<sub>4</sub> that was pre-sorbed with phosphate (PO<sub>4</sub>) to test the effect of U(IV) speciation on the isotopic fractionation. In fact, the sorption of PO<sub>4</sub> is known to favor the production of non-crystalline U(IV) species rather than UO<sub>2</sub> species as in the case of pure Fe<sub>3</sub>O<sub>4</sub>.

We also conducted two adsorption experiments at different U loading to assess how U(VI) adsorption contributes to the total fractionation effect. We used maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) as a proxy for non-reactive Fe<sub>3</sub>O<sub>4</sub>.  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> has the same structure as Fe<sub>3</sub>O<sub>4</sub>, but it does not contain Fe(II), and it is no capable of reducing U(VI).

#### 1.6 References

(1) Langmuir, D. Uranium Solution-Mineral Equilibria at Low Temperatures with Applications to Sedimentary Ore Deposits. *Geochimica et Cosmochimica Acta* **1978**, *42* (6, Part A), 547–569. https://doi.org/10.1016/0016-7037(78)90001-7.

Newsome, L.; Morris, K.; Lloyd, J. R. The Biogeochemistry and Bioremediation of Uranium and
 Other Priority Radionuclides. *Chemical Geology* 2014, 363, 164–184.
 https://doi.org/10.1016/j.chemgeo.2013.10.034.

(3) Lovley, D.; Phillips, E.; Gorby, Y.; Landa, E. Microbial Reduction of Uranium. *Nature* **1991**, *350* (6317), 413–416. https://doi.org/10.1038/350413a0.

(4) Structure of Biogenic Uraninite Produced by Shewanella oneidensis Strain MR-1 - Environmental Science & Technology (ACS Publications) https://pubs.acs.org/doi/abs/10.1021/es800579g (accessed Oct 27, 2018).

(5) 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. Non-Uraninite Products of Microbial U(VI) Reduction. *Environ. Sci. Technol.* 2010, 44 (24), 9456–9462. https://doi.org/10.1021/es101675a.

(6) Bargar, J. R.; Williams, K. H.; Campbell, K. M.; Long, P. E.; Stubbs, J. E.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Alessi, D. S.; Stylo, M.; Webb, S. M.; et al. Uranium Redox Transition Pathways in Acetate-Amended Sediments. *PNAS* **2013**, *110* (12), 4506–4511. https://doi.org/10.1073/pnas.1219198110.

(7) Morin, G.; Mangeret, A.; Othmane, G.; Stetten, L.; Seder-Colomina, M.; Brest, J.; Ona-Nguema, G.; Bassot, S.; Courbet, C.; Guillevic, J.; et al. Mononuclear U(IV) Complexes and Ningyoite as Major Uranium Species in Lake Sediments. *Geochemical Perspectives Letters* **2016**. https://doi.org/10.7185/geochem-let.1610.

Wang, Y.; Bagnoud, A.; Suvorova, E.; McGivney, E.; Chesaux, L.; Phrommavanh, V.; Descostes, M.;
 Bernier-Latmani, R. Geochemical Control on Uranium(IV) Mobility in a Mining-Impacted Wetland. *Environ. Sci. Technol.* 2014, 48 (17), 10062–10070. https://doi.org/10.1021/es501556d.

16
(9) Cerrato, J. M.; Ashner, M. N.; Alessi, D. S.; Lezama-Pacheco, J. S.; Bernier-Latmani, R.; Bargar, J. R.;
 Giammar, D. E. Relative Reactivity of Biogenic and Chemogenic Uraninite and Biogenic Noncrystalline
 U(IV). *Environ. Sci. Technol.* 2013, 47 (17), 9756–9763. https://doi.org/10.1021/es401663t.

(10) Lovley, D. R.; Phillips, E. J. P.; Gorby, Y. A.; Landa, E. R. Microbial Reduction of Uranium. *Nature* **1991**, *350* (6317), 413. https://doi.org/10.1038/350413a0.

(11) Veeramani, H.; Alessi, D. S.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Sharp, J. O.; Dippon, U.; Kappler, A.; Bargar, J. R.; Bernier-Latmani, R. Products of Abiotic U(VI) Reduction by Biogenic Magnetite and Vivianite. *Geochimica et Cosmochimica Acta* **2011**, *75* (9), 2512–2528. https://doi.org/10.1016/j.gca.2011.02.024.

 (12) Veeramani, H.; Scheinost, A. C.; Monsegue, N.; Qafoku, N. P.; Kukkadapu, R.; Newville, M.; Lanzirotti, A.; Pruden, A.; Murayama, M.; Hochella, M. F. Abiotic Reductive Immobilization of U(VI) by Biogenic Mackinawite. *Environmental Science & Technology* **2013**, *47* (5), 2361–2369. https://doi.org/10.1021/es304025x.

(13) Williams, K. H.; Bargar, J. R.; Lloyd, J. R.; Lovley, D. R. Bioremediation of Uranium-Contaminated Groundwater: A Systems Approach to Subsurface Biogeochemistry. *Current Opinion in Biotechnology* **2013**, *24* (3), 489–497. https://doi.org/10.1016/j.copbio.2012.10.008.

 (14) Schofield, E. J.; Veeramani, H.; Sharp, J. O.; Suvorova, E.; Bernier-Latmani, R.; Mehta, A.; Stahlman, J.; Webb, S. M.; Clark, D. L.; Conradson, S. D.; et al. Structure of Biogenic Uraninite Produced by *Shewanella Oneidensis* Strain MR-1. *Environmental Science & Technology* 2008, 42 (21), 7898–7904. https://doi.org/10.1021/es800579g.

(15) Stylo, M.; Alessi, D. S.; Shao, P. P.; Lezama-Pacheco, J. S.; Bargar, J. R.; Bernier-Latmani, R. Bioge-ochemical Controls on the Product of Microbial U(VI) Reduction. *Environmental Science & Technology* 2013, 47 (21), 12351–12358. https://doi.org/10.1021/es402631w.

(16) Suzuki, Y.; Kelly, S. D.; Kemner, K. M.; Banfield, J. F. Radionuclide Contamination: Nanometre-Size Products of Uranium Bioreduction. *Nature* **2002**, *419* (6903), 134–134. https://doi.org/10.1038/419134a.

(17) Sharp, J. O.; Schofield, E. J.; Veeramani, H.; Suvorova, E. I.; Kennedy, D. W.; Marshall, M. J.; Mehta,A.; Bargar, J. R.; Bernier-Latmani, R. Structural Similarities between Biogenic Uraninites Produced by

Phylogenetically and Metabolically Diverse Bacteria. *Environ. Sci. Technol.* **2009**, *43* (21), 8295–8301. https://doi.org/10.1021/es901281e.

(18) Campbell, K. M.; Veeramani, H.; Urich, K.-U.; Blue, L. Y.; Giammar, D. E.; Bernier-Latmani, R.; Stubbs, J. E.; Suvorova, E.; Yabusaki, S.; Lezama-Pacheco, J. S.; et al. Oxidative Dissolution of Biogenic Uraninite in Groundwater at Old Rifle, CO. *Environ. Sci. Technol.* **2011**, *45* (20), 8748–8754. https://doi.org/10.1021/es200482f.

(19) Bargar, J. R.; Williams, K. H.; Campbell, K. M.; Long, P. E.; Stubbs, J. E.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Alessi, D. S.; Stylo, M.; Webb, S. M.; et al. Uranium Redox Transition Pathways in Acetate-Amended Sediments. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (12), 4506–4511. https://doi.org/10.1073/pnas.1219198110.

(20) Alessi, D. S.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Janousch, M.; Bargar, J. R.; Persson, P.; Bernier-Latmani, R. The Product of Microbial Uranium Reduction Includes Multiple Species with U(IV)–Phosphate Coordination. *Geochimica et Cosmochimica Acta* 2014, 131, 115–127. https://doi.org/10.1016/j.gca.2014.01.005.

(21) Alessi, D. S.; Lezama-Pacheco, J. S.; Janot, N.; Suvorova, E. I.; Cerrato, J. M.; Giammar, D. E.; Davis, J. A.; Fox, P. M.; Williams, K. H.; Long, P. E.; et al. Speciation and Reactivity of Uranium Products Formed during in Situ Bioremediation in a Shallow Alluvial Aquifer. *Environ. Sci. Technol.* **2014**, *48* (21), 12842–12850. https://doi.org/10.1021/es502701u.

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. Non-Uraninite Products of Microbial U(VI) Reduction. *Environ. Sci. Technol.* 2010, 44 (24), 9456–9462. https://doi.org/10.1021/es101675a.

(23) Bargar, J. R.; Bernier-Latmani, R.; Giammar, D. E.; Tebo, B. M. Biogenic Uraninite Nanoparticles and Their Importance for Uranium Remediation. *Elements* **2008**, *4* (6), 407–412. https://doi.org/10.2113/gselements.4.6.407.

(24) Kelly, S. D.; Kemner, K. M.; Carley, J.; Criddle, C.; Jardine, P. M.; Marsh, T. L.; Phillips, D.; Watson,
D.; Wu, W.-M. Speciation of Uranium in Sediments before and after In Situ Biostimulation. *Environ. Sci. Technol.* 2008, 42 (5), 1558–1564. https://doi.org/10.1021/es071764i.

(25) Stetten, L.; Mangeret, A.; Brest, J.; Seder-Colomina, M.; Le Pape, P.; Ikogou, M.; Zeyen, N.; Thouvenot, A.; Julien, A.; Alcalde, G.; et al. Geochemical Control on the Reduction of U(VI) to Mononuclear U(IV) Species in Lacustrine Sediments. *Geochimica et Cosmochimica Acta* **2018**, *222* (Supplement C), 171–186. https://doi.org/10.1016/j.gca.2017.10.026.

(26) Latta, D. E.; Mishra, B.; Cook, R. E.; Kemner, K. M.; Boyanov, M. I. Stable U(IV) Complexes Form at High-Affinity Mineral Surface Sites. *Environmental Science & Technology* **2014**, *48* (3), 1683–1691. https://doi.org/10.1021/es4047389.

(27) Ulrich, K.-U.; Ilton, E. S.; Veeramani, H.; Sharp, J. O.; Bernier-Latmani, R.; Schofield, E. J.; Bargar, J. R.; Giammar, D. E. Comparative Dissolution Kinetics of Biogenic and Chemogenic Uraninite under Oxidizing Conditions in the Presence of Carbonate. *Geochimica et Cosmochimica Acta* **2009**, *73* (20), 6065–6083. https://doi.org/10.1016/j.gca.2009.07.012.

Kelly, S. D.; Wu, W.-M.; Yang, F.; Criddle, C. S.; Marsh, T. L.; O'Loughlin, E. J.; Ravel, B.; Watson, D.;
 Jardine, P. M.; Kemner, K. M. Uranium Transformations in Static Microcosms. *Environ. Sci. Technol.* 2010, 44 (1), 236–242. https://doi.org/10.1021/es902191s.

(29) Newsome, L.; Morris, K.; Shaw, S.; Trivedi, D.; Lloyd, J. R. The Stability of Microbially Reduced U(IV); Impact of Residual Electron Donor and Sediment Ageing. *Chemical Geology* **2015**, *409*, 125–135. https://doi.org/10.1016/j.chemgeo.2015.05.016.

Bhattacharyya, A.; Campbell, K. M.; Kelly, S. D.; Roebbert, Y.; Weyer, S.; Bernier-Latmani, R.; Borch,
 T. Biogenic Non-Crystalline U<sup>(IV)</sup> Revealed as Major Component in Uranium Ore Deposits. *Nature Communications* 2017, *8*, 15538. https://doi.org/10.1038/ncomms15538.

(31) Cumberland, S. A.; Douglas, G.; Grice, K.; Moreau, J. W. Uranium Mobility in Organic Matter-Rich Sediments: A Review of Geological and Geochemical Processes. *Earth-Science Reviews* **2016**, *159*, 160–185. https://doi.org/10.1016/j.earscirev.2016.05.010.

Newsome, L.; Morris, K.; Shaw, S.; Trivedi, D.; Lloyd, J. R. The Stability of Microbially Reduced U(IV);
 Impact of Residual Electron Donor and Sediment Ageing. *Chemical Geology* 2015, *409* (Supplement C), 125–135. https://doi.org/10.1016/j.chemgeo.2015.05.016.

Borch, T.; Kretzschmar, R.; Kappler, A.; Cappellen, P. V.; Ginder-Vogel, M.; Voegelin, A.; Campbell,
K. Biogeochemical Redox Processes and Their Impact on Contaminant Dynamics. *Environ. Sci. Technol.*2010, 44 (1), 15–23. https://doi.org/10.1021/es9026248.

(34) Ulrich, K.-U.; Ilton, E. S.; Veeramani, H.; Sharp, J. O.; Bernier-Latmani, R.; Schofield, E. J.; Bargar, J. R.; Giammar, D. E. Comparative Dissolution Kinetics of Biogenic and Chemogenic Uraninite under Oxidizing Conditions in the Presence of Carbonate. *Geochim. Cosmochim. Acta* **2009**, *73* (20), 6065–6083. https://doi.org/10.1016/j.gca.2009.07.012.

(35) Bi, Y.; Hyun, S. P.; Kukkadapu, R. K.; Hayes, K. F. Oxidative Dissolution of UO2 in a Simulated Groundwater Containing Synthetic Nanocrystalline Mackinawite. *Geochimica et Cosmochimica Acta* **2013**, *102*, 175–190. https://doi.org/10.1016/j.gca.2012.10.032.

(36) Abdelouas, A.; Lutze, W.; Nuttall, H. E. Oxidative Dissolution of Uraninite Precipitated on Navajo Sandstone. *Journal of Contaminant Hydrology* **1999**, *36* (3), 353–375. https://doi.org/10.1016/S0169-7722(98)00151-X.

(37) Carpenter, J.; Bi, Y.; Hayes, K. F. Influence of Iron Sulfides on Abiotic Oxidation of UO2 by Nitrite and Dissolved Oxygen in Natural Sediments. *Environ. Sci. Technol.* **2015**, *49* (2), 1078–1085. https://doi.org/10.1021/es504481n.

(38) Bi, Y.; Hayes, K. F. Nano-FeS Inhibits UO2 Reoxidation under Varied Oxic Conditions. *Environ. Sci. Technol.* **2014**, *48* (1), 632–640. https://doi.org/10.1021/es4043353.

(39) Bi, Y.; Hayes, K. F. Surface Passivation Limited UO2 Oxidative Dissolution in the Presence of FeS. *Environ. Sci. Technol.* **2014**, *48* (22), 13402–13411. https://doi.org/10.1021/es5041392.

(40) Bi, Y.; Stylo, M.; Bernier-Latmani, R.; Hayes, K. F. Rapid Mobilization of Noncrystalline U(IV) Coupled with FeS Oxidation. *Environ. Sci. Technol.* **2016**, *50* (3), 1403–1411. https://doi.org/10.1021/acs.est.5b04281.

(41) Sharp, J. O.; Lezama-Pacheco, J. S.; Schofield, E. J.; Junier, P.; Ulrich, K.-U.; Chinni, S.; Veeramani, H.; Margot-Roquier, C.; Webb, S. M.; Tebo, B. M.; et al. Uranium Speciation and Stability after Reductive Immobilization in Aquifer Sediments. *Geochimica et Cosmochimica Acta* **2011**, *75* (21), 6497–6510. https://doi.org/10.1016/j.gca.2011.08.022.

(42) Jeong, H. Y.; Han, Y.-S.; Hayes, K. F. X-Ray Absorption and X-Ray Photoelectron Spectroscopic Study of Arsenic Mobilization during Mackinawite (FeS) Oxidation. *Environ. Sci. Technol.* **2010**, *44* (3), 955–961. https://doi.org/10.1021/es902577y.

(43) Jeong, H. Y.; Han, Y.-S.; Park, S. W.; Hayes, K. F. Aerobic Oxidation of Mackinawite (FeS) and Its Environmental Implication for Arsenic Mobilization. *Geochimica et Cosmochimica Acta* **2010**, *74* (11), 3182–3198. https://doi.org/10.1016/j.gca.2010.03.012.

(44) Yuan, X.; Nico, P. S.; Huang, X.; Liu, T.; Ulrich, C.; Williams, K. H.; Davis, J. A. Production of Hydrogen Peroxide in Groundwater at Rifle, Colorado. *Environ. Sci. Technol.* **2017**, *51* (14), 7881–7891. https://doi.org/10.1021/acs.est.6b04803.

(45) Vermilyea, A. W.; Hansard, S. P.; Voelker, B. M. Dark Production of Hydrogen Peroxide in the Gulf of Alaska. *Limnology and Oceanography 55* (2), 580–588. https://doi.org/10.4319/lo.2010.55.2.0580.

(46) Murphy, S. A.; Solomon, B. M.; Meng, S.; Copeland, J. M.; Shaw, T. J.; Ferry, J. L. Geochemical Production of Reactive Oxygen Species From Biogeochemically Reduced Fe. *Environ. Sci. Technol.* **2014**, *48* (7), 3815–3821. https://doi.org/10.1021/es4051764.

(47) Cheng, D.; Yuan, S.; Liao, P.; Zhang, P. Oxidizing Impact Induced by Mackinawite (FeS) Nanoparticles at Oxic Conditions Due to Production of Hydroxyl Radicals. *Environ. Sci. Technol.* **2016**, *50* (21), 11646–11653. https://doi.org/10.1021/acs.est.6b02833.

(48) Tong, M.; Yuan, S.; Ma, S.; Jin, M.; Liu, D.; Cheng, D.; Liu, X.; Gan, Y.; Wang, Y. Production of Abundant Hydroxyl Radicals from Oxygenation of Subsurface Sediments. *Environ. Sci. Technol.* **2016**, *50* (1), 214–221. https://doi.org/10.1021/acs.est.5b04323.

(49) Page, S. E.; Sander, M.; Arnold, W. A.; McNeill, K. Hydroxyl Radical Formation upon Oxidation of Reduced Humic Acids by Oxygen in the Dark. *Environ. Sci. Technol.* **2012**, *46* (3), 1590–1597. https://doi.org/10.1021/es203836f.

(50) Pham, A. N.; Xing, G.; Miller, C. J.; Waite, T. D. Fenton-like Copper Redox Chemistry Revisited: Hydrogen Peroxide and Superoxide Mediation of Copper-Catalyzed Oxidant Production. *Journal of Catalysis* **2013**, *301*, 54–64. https://doi.org/10.1016/j.jcat.2013.01.025.

(51) Learman, D. R.; Voelker, B. M.; Vazquez-Rodriguez, A. I.; Hansel, C. M. Formation of Manganese Oxides by Bacterially Generated Superoxide. *Nature Geoscience* **2011**, *4* (2), 95–98. https://doi.org/10.1038/ngeo1055.

(52) Andersen, M. B.; Stirling, C. H.; Weyer, S. Uranium Isotope Fractionation. *Reviews in Mineralogy and Geochemistry* **2017**, *82* (1), 799–850. https://doi.org/10.2138/rmg.2017.82.19.

(53) Weyer, S.; Anbar, A. D.; Gerdes, A.; Gordon, G. W.; Algeo, T. J.; Boyle, E. A. Natural Fractionation of 238U/235U. *Geochimica et Cosmochimica Acta* 2008, 72 (2), 345–359. https://doi.org/10.1016/j.gca.2007.11.012.

(54) Brennecka, G. A.; Wasylenki, L. E.; Bargar, J. R.; Weyer, S.; Anbar, A. D. Uranium Isotope Fractionation during Adsorption to Mn-Oxyhydroxides. *Environmental Science & Technology* **2011**, *45* (4), 1370– 1375. https://doi.org/10.1021/es103061v.

(55) Jemison, N. E.; Johnson, T. M.; Shiel, A. E.; Lundstrom, C. C. Uranium Isotopic Fractionation Induced by U(VI) Adsorption onto Common Aquifer Minerals. *Environ. Sci. Technol.* **2016**, *50* (22), 12232– 12240. https://doi.org/10.1021/acs.est.6b03488.

(56) Chen, X.; Romaniello, S. J.; Herrmann, A. D.; Wasylenki, L. E.; Anbar, A. D. Uranium Isotope Fractionation during Coprecipitation with Aragonite and Calcite. *Geochimica et Cosmochimica Acta* **2016**, *188*, 189–207. https://doi.org/10.1016/j.gca.2016.05.022.

(57) Wang, X.; Johnson, T. M.; Lundstrom, C. C. Isotope Fractionation during Oxidation of Tetravalent Uranium by Dissolved Oxygen. *Geochimica et Cosmochimica Acta* **2015**, *150*, 160–170. https://doi.org/10.1016/j.gca.2014.12.007.

(58) Stylo, M.; Neubert, N.; Wang, Y.; Monga, N.; Romaniello, S. J.; Weyer, S.; Bernier-Latmani, R. Uranium Isotopes Fingerprint Biotic Reduction. *PNAS* 2015, *112* (18), 5619–5624.
https://doi.org/10.1073/pnas.1421841112.

(59) Basu, A.; Sanford, R. A.; Johnson, T. M.; Lundstrom, C. C.; Löffler, F. E. Uranium Isotopic Fractionation Factors during U(VI) Reduction by Bacterial Isolates. *Geochimica et Cosmochimica Acta* **2014**, *136*, 100–113. https://doi.org/10.1016/j.gca.2014.02.041.

Brown, S. T.; Basu, A.; Ding, X.; Christensen, J. N.; DePaolo, D. J. Uranium Isotope Fractionation by
Abiotic Reductive Precipitation. *PNAS* 2018, 115 (35), 8688–8693.
https://doi.org/10.1073/pnas.1805234115.

 Bopp, C. J.; Lundstrom, C. C.; Johnson, T. M.; Sanford, R. A.; Long, P. E.; Williams, K. H. Uranium <sup>238</sup>
 U/ <sup>235</sup> U Isotope Ratios as Indicators of Reduction: Results from an in Situ Biostimulation Experiment at Rifle, Colorado, U.S.A. *Environmental Science & Technology* **2010**, *44* (15), 5927–5933. https://doi.org/10.1021/es100643v.

(62) Noël, V.; Boye, K.; Kukkadapu, R. K.; Bone, S.; Lezama Pacheco, J. S.; Cardarelli, E.; Janot, N.; Fendorf, S.; Williams, K. H.; Bargar, J. R. Understanding Controls on Redox Processes in Floodplain Sediments of the Upper Colorado River Basin. *Science of The Total Environment* **2017**, *603–604*, 663–675. https://doi.org/10.1016/j.scitotenv.2017.01.109.

Moon, H. S.; McGuinness, L.; Kukkadapu, R. K.; Peacock, A. D.; Komlos, J.; Kerkhof, L. J.; Long, P.
 E.; Jaffé, P. R. Microbial Reduction of Uranium under Iron- and Sulfate-Reducing Conditions: Effect of Amended Goethite on Microbial Community Composition and Dynamics. *Water Research* 2010, 44 (14), 4015–4028. https://doi.org/10.1016/j.watres.2010.05.003.

(64) Anderson, R. T.; Vrionis, H. A.; Ortiz-Bernad, I.; Resch, C. T.; Long, P. E.; Dayvault, R.; Karp, K.; Marutzky, S.; Metzler, D. R.; Peacock, A.; et al. Stimulating the In Situ Activity of Geobacter Species To Remove Uranium from the Groundwater of a Uranium-Contaminated Aquifer. *Appl. Environ. Microbiol.* 2003, *69* (10), 5884–5891. https://doi.org/10.1128/AEM.69.10.5884-5891.2003.

## Chapter 2 The effect of aging on the structure of non-crystalline tetravalent uranium

This chapter presents the results of a series of column experiments using Rifle sediments to mimic a bioremediation scenario and immobilize bioreduced species of U(IV) within the sediments. The characterization of the sediments after the bioreduction phase is presented here. In addition, we present the speciation of bioreduced U(IV) species over time during an aging period of 1 year in anoxic batch experiments. The speciation was probed by X-ray absorption spectroscopy.

This chapter is presented as a manuscript to be submitted for publication in Environmental Science and Technology.

Supporting information of this manuscript is presented in Annex 1.

*Luca Loreggian* - experimental work and associated wet chemistry (ICP-MS, ICP-OES, IC) and mineralogical (XAS) analysis; *Agnes Novotny, Sophie Bretagne* - maintenance of the column experiments, sampling of the column experiments, and measurements of Fe(II); *Yuheng Wang* - XAS modelling review, manuscript review; *Rizlan Bernier-Latmani* - conceptual guidance and extensive manuscript review.

## 2.1 Abstract

Reductive bioremediation of uranium has been largely explored as a decontamination strategy. Via the *in-situ* biostimulation of microbial processes in the subsurface, hexavalent U is reduced to less soluble tetravalent species which are immobilized within the sediments. Although, the mineral uraninite (UO<sub>2</sub>) was initially considered the dominant product of biological reduction, non-crystalline U(IV) species (NCU4) are found to be abundant upon bioreduction in the environment. Whilst UO<sub>2</sub> is likely to be stable under various geochemical conditions, NCU4 is susceptible to oxidation and remobilization. However, it has been recently proposed that, through aging, NCU4 might transform to UO<sub>2</sub>, a transformation which potentially enhances the stability of NCU4. In this study, we perform laboratory experiments to produce NCU4 species in natural sediments mimicking the environmental conditions during a bioremediation intervention. We use X-ray absorption spectroscopy (XAS) to systematically monitor the coordination environment of NCU4 species through an aging period of 12 months under stable anoxic and dark conditions in static microcosms. XAS revealed that, under the investigated conditions, the speciation of U(IV) does not change over time. Thus, because NCU4 is the dominant species in the sediments, bioreduced U(IV) species remain vulnerable to oxidation and remobilization to the aqueous phase even after a 12-month aging period.

KEYWORDS: Amorphous tetravalent uranium, aging effect, transformation, X-ray absorption spectroscopy

## 2.2 Introduction

Since the discovery of microbial uranium (U) reduction<sup>1</sup>, *in-situ* bioremediation has been proposed as an efficient and economical strategy to mitigate U contamination in the subsurface. Bioremediation aims to stimulate the microbial activity of indigenous microorganisms so that, when reducing conditions are established, mobile hexavalent U (U(VI)) is reduced to less soluble tetravalent U (U(IV)). The latter precipitates and is immobilized in the sediments. In the past, the mineral uraninite  $(UO_2)$  was considered to be the primary product of biological reduction. Because of its crystalline structure and relative resistance to oxidation, UO<sub>2</sub> is likely to be stable for an extended period of time under various geochemical conditions and, therefore, is the most desirable product of bioreduction. However, field studies<sup>2-4</sup> and laboratory work<sup>5,6</sup> revealed that non-crystalline U(IV) species (NCU4) are more abundant than UO<sub>2</sub> upon bioreduction. XAS studies reported that NCU4 is coordinated with carboxyl or phosphate ligands<sup>7,8</sup> that are proposed to hinder the nucleation of UO<sub>2</sub> and favor NCU4 formation<sup>8</sup>. Because NCU4 has an amorphous structure<sup>5</sup>, higher reactivity<sup>9</sup>, lower tendency to form aggregates<sup>10</sup>, and a number of chemical compositions<sup>7</sup>, it responds differently to changes in redox conditions and appears to be more labile than  $UO_2^{11,12}$ . NCU4 is ubiquitous in bioreduced environments<sup>2,4,13</sup> including naturally reduced zones<sup>14–16</sup>. Therefore, it is a highly relevant species of U(IV) in the environment. Before promoting bioremediation as an effective strategy for U immobilization, more work is required to characterize NCU4 stability in the subsurface.

Few studies have investigated the potential transformation of NCU4 to UO<sub>2</sub> and the consequential improved resistance to oxidation by oxygen and to ligand-promoted dissolution. Two static microcosm experiments<sup>17,18</sup> used X-ray absorption spectroscopy (XAS) to document structural changes in U(IV) speciation after 11 and 15 months of incubation. The relative amount of UO<sub>2</sub> increased over time in the system, and it was concluded that freshly-reduced NCU4 might potentially age to crystalline UO<sub>2</sub>. However, when aged U(IV) was challenged in a re-oxidation experiment, it did not show a higher resistance to oxidation than NCU4, as would have been expected had  $UO_2$  been produced<sup>18</sup>. Hence, there is some uncertainty as to the extent of the transformation of NCU4 to  $UO_2$  through aging.

This uncertainty is exacerbated by findings from an investigation of U speciation in field-deployed column experiments. Using XAS and chemical extraction, Bargar et al. (2013) reported that the fractions of NCU4 and recalcitrant U(IV), assumed to be UO<sub>2</sub>, remained unchanged in fresh and aged samples<sup>2</sup>. Rather than attributing UO<sub>2</sub> formation to the aging of NCU4, Bargar et *al.* (2013) proposed that UO<sub>2</sub> was the end product of abiotic reduction of U(VI), which in a complex system such as natural sediments, occurs concomitantly with biological U(VI) reduction. Therefore, when relatively high UO<sub>2</sub> contributions are found in naturally reduced zones, two mechanisms are invoked: either the establishment of specific local conditions favoring UO<sub>2</sub> precipitation (i.e., a decrease in ligand concentration<sup>15,20</sup>) or the preferential oxidation of highly reactive phases (i.e., NCU4) and subsequent enrichment of more recalcitrant uraninite<sup>15</sup>. Given the complexity of the natural environment where both biotic and abiotic processes can contribute to immobilizing U(IV), it is difficult to determine whether UO<sub>2</sub> is the end product of abiotic reduction or NCU4-aging. However, as NCU4 was found to be the predominant species in some pristine environments<sup>14</sup>, its persistence as an end product of U(VI) reduction appears plausible.

The objective of this study is to monitor the speciation and coordination environment of sedimentproduced U(IV) over a period of 12 months. To that end, we conducted a series of biostimulated flowthrough column experiments with sediments collected in a former U processing site in Rifle, CO (USA) in order to accumulate NCU4. The freshly bioreduced sediments harboring U(IV) were incubated in static microcosms under anoxic and dark conditions for 12 months, and the coordination environment of U probed every four months.

## 2.3 Materials and methods

#### 2.3.1 Description of the sediments

The sediments used in all the experiments were collected in the background area of a former U mining and milling processing site at Rifle, CO (USA), in the Colorado River Basin. This site was designated as the Old Rifle site, and it is part of the Uranium Mill Tailings Remedial Action (UMTRA) program of the U.S. Department of energy<sup>10</sup>. At Old Rifle, groundwater is confined in the topmost layer by a relatively impermeable silty shale layer at ~6 m depth<sup>10,21</sup>. The groundwater flows towards the Colorado River at ~0.82 m/day<sup>10</sup>. The concentration of dissolved oxygen ranges from 0 to 0.6 mg/L, and its average is 0.2 mg/L<sup>22</sup>. U concentration varies between 0.4 and 1.8  $\mu$ M<sup>23</sup>. In general, the aquifer has a high concentration of sulfate (SO<sub>4</sub><sup>2-</sup>) (9-16 mM)<sup>24,25</sup> and high alkalinity (~10 meg/L)<sup>26</sup>.

The sediments sampled at Old Rifle were designated as Rifle area background sediment (RABS)<sup>27</sup>. RABS were dried, sieved (<2 mm) and stored in the dark until use. In this study, Rifle artificial groundwater (RAGW) was prepared to mimic conditions in the field. RAGW composition was designed based on Rifle groundwater composition measured in previous studies<sup>23,25</sup>.

#### 2.3.2 Design and operation of the column experiments

Three borosilicate glass chromatography columns (from Kimble Kontes, Vineland, NJ) measuring 15 cm in length and 5 cm in diameter were used in flow-through mode. The columns are supplied with 20  $\mu$ m mesh high density polyethylene (HDPE) bed supports at both top and bottom ends. To avoid biofilm growth and loss of uranium within the bed support, the bottom one was removed and a polyethylene sulfonate (PES) filter (0.22  $\mu$ m) was added at the inlet of the column to prevent biofilm growth in the inlet tubing. The columns were wet packed with ~450 g of RABS per column and artificial RAGW. RAGW was prepared anoxically by purging the solution with CO<sub>2</sub>/N<sub>2</sub> gas (20:80) in glass bottles and autoclaved. The RAGW was injected upwards through the columns at a constant flow rate of 2 mL/h to mimic the natural groundwater flow at the Old Rifle site using a peristaltic pump (Ismatec IP, Glattbrugg, Switzerland). Viton tubings and polypropylene connectors were used to connect the system.

To stimulate the microbial activity, RAGW was amended with: 1.5 g/L of yeast extract (Bacto, USA), 10.5 mg/L of glycerol (Sigma Aldrich, Germany) and 1.5 g/L of molasses (EcoMolasses, International Molasses Itd USA). All three column experiments (columns 1 through 3) included 14 mM sulfate in the influent to favor sulfate-reducing conditions (SRC).

The three SRC columns were biostimulated for 97 days before adding U to RAGW to start the U(VI) bioreduction phase. The U(VI) bioreduction phase was initiated at day 97 by adding 12.5 µM uranyl-acetate (Fluka, Switzerland) that was filter-sterilized through a 0.22 µm poresize PTFE filter (ThermoFisher, USA) to the sterile RAGW and electron donor mixture. However, biofilm growth in the tubing and U(VI) adsorption onto the PES filter resulted in partial loss of U before the inlet of the columns. Therefore, on day 201, the experiment was interrupted to improve the setup. Subsequently, U(VI) was added as a separate solution containing only 1 mM bicarbonate. The RAGW+electron donors and U solutions were pumped at the same flow rate and mixed in a 1:1 ratio immediately upstream of the column inlet by a T-connector. The influent solution was periodically sampled after the T-connector to verify that sufficient mixing occurred and that no U was lost. Furthermore, to minimize the impact of biofilm formation and clogging, tubings and filters were changed every ~2 weeks. Finally, the PES filter at the inlet of each column was substituted with a PTFE filter to minimize U adsorption.

U concentration in the influent was gradually increased, first to 20  $\mu$ M at day 201 and later to 50  $\mu$ M at day 285. The columns were operated for 407 days. At that point, it was deemed that a sufficient amount of U had been accumulated for subsequent experiments.

At the end of the bioreduction phase, the columns were transferred to an anaerobic chamber (MBraun, Germany) with an atmosphere of 20%:80% CO<sub>2</sub>:N<sub>2</sub>. SRC columns 1, 2 and 3ere destructively sampled. The sediments were divided into eight sections along the column's length. Each of these sections was dried under vacuum in a desiccator in the anaerobic chamber and homogenized prior to further processing.

#### 2.3.3 Characterization of the effluent composition

The column effluent was routinely sampled for analysis of its composition throughout the experiment. Water samples were filtered through 0.22 µm PTFE filters before analysis for total U, total Fe, and SO<sub>4</sub><sup>2-</sup> concentration. Total U in the influent and effluent was analyzed using inductively-coupled plasma mass spectrometry (ICP-MS; Perkin Elmer ELAN DRC II). Total Fe was measured in the effluents by Inductively-coupled plasma optical emission spectrometry (ICP-OES; Multitype ICP Emission Spectrometer, ICPE-9000, Shimadzu). Fe(II) was preserved from rapid oxidation by collecting 0.5 mL of effluent solution directly into 0.5 mL of 1 N HCl. The concentration of Fe(II) was determined photometrically on filtered samples with the Ferrozine method<sup>28</sup>. Sulfate remaining in the effluent was analyzed with Ion Chromatography (IC) (System ICS-3000 Dionex).

#### 2.3.4 Chemistry of the sediments

The concentration of major and trace elements in the sediments was measured by X-ray fluorescence spectrometry with a PANalytical Axio-mAX spectrometer. Briefly, the analysis was conducted on ground, dried and homogenized samples that were prepared by pressing a minimum of 4 g of powder with Hoechst-wax-C in disks. The limit of detection depends on the element, and it ranges between 1 and 7 ppm. The accuracy of the instrument was verified with standard reference materials.

#### 2.3.5 X-ray absorption spectroscopy (XAS)

XAS was used to determine the speciation of U, Fe, and S in the sediments harvested from the bottom section of the column experiments because it contains the highest concentration of U.

Uranium L<sub>III</sub>-edge XAS data were collected in fluorescence mode at beamline (BL) 11-2 of the Stanford Synchrotron Radiation Lightsource (SSRL) at Stanford Linear Accelerator Laboratory (SLAC) (Menlo Park, CA) and BL I20-scanning at Diamond Light Source (DLS) (Didcot, UK). The fluorescence signal was collected with a 100-element Germanium (Ge) detector (Canberra Industries, USA) at SSRL and with a 64-element Ge detector (Canberra Industries, USA) at DLS. The dried sediments were ground and pressed into 7 mm diameter pellets protected by Kapton tape and mounted on aluminum holders for measurement at BL 11-2 and in Nalgene® cryovials for measurements at BL I-20. Sample preparation was conducted in an anaerobic chamber (Coy Laboratory Products Inc, USA) with an atmosphere of 3%:97% H<sub>2</sub>:N<sub>2</sub>. Samples were shipped to SSRL and DLS in a hermetically sealed stainless-steel shipping canister (Schuett-Biotec GmbH, Gottingen, Germany). XAS analysis was conducted at 77K using a liquid nitrogen (LN2) cryostat to reduce beam damage, preserve air-sensitive samples from oxidation, and improve data quality. The energy selection was achieved by a 30% detuned double-crystal Si (220) monochromator at BL 11-2 and Si(111) at BL I20-scanning. The calibration of the energy was performed on the first inflection point of yttrium (Y) foil reference (17,038 eV), that was placed between the  $I_1$  and  $I_2$  ion chambers. The same Y foil was used as a calibrant by collecting its transmission signal in I<sub>2</sub> simultaneously with the samples analyzed for the entire duration of the experiment. Multiple scans were required for each sample. Scans were merged to reduce noise and achieve an adequate signal-to-noise ratio.

Iron (Fe) K-edge XAS measurements were performed at BL 4-1 of SSRL at SLAC. Spectra were collected in transmission mode at 77K in an LN2 cryostat. The energy was selected by a Si(111) double crystal mono-chromator. The energy was calibrated on the first inflection point of a Fe foil (7,112 eV). The dried and

ground samples were diluted in fructose for optimal signal in transmission mode and pressed in 7 mm diameter pellets that were protected in Kapton tape and mounted in aluminum holders. The samples were always handled under anoxic conditions.

Sulfur (S) K-edge XAS measurements were conducted in fluorescence mode on ground sediments at BL 4-3 at SSRL. A Si(111) double crystal monochromator was used for energy selection. Ground samples were loaded on sulfur-free tape and placed into aluminum holders. The samples were maintained under anoxic conditions and loaded on site in an anaerobic chamber with a similar atmosphere to what previously indicated. Measurements were conducted in a helium atmosphere at room temperature in fluorescence mode. The fluorescence signal was collected using a passivated implanted planar silicon detector (PIPS, Canberra) measuring ~10 cm in diameter for concentrated samples (>1 wt. %). The energy for S K-edge was calibrated at the first inflection point of a sodium thiosulfate standard (2,472 eV). In order to protect the samples from beam damage, shutters were used to limit sample beam exposure between collection times.

#### 2.3.6 XAS Data Analysis

Depending on the concentration of the element of interest, multiple scans were needed for optimal signalto-noise ratio. U L<sub>III</sub> edge XAS data collected with the Ge detector were processed with the SixPACK software for screening single spectra from each Ge element<sup>29</sup>. U, Fe, and S XAS data were averaged, normalized and background-subtracted using Athena<sup>29</sup> to extract experimental XANES and EXAFS functions.

U L<sub>III</sub>-edge XANES, S K-edge XANES, and Fe K-edge EXAFS spectra were analyzed by linear combination fit analysis (LCF) with reference compounds that are reported elsewhere (Figure SI-1, Figure SI-2, and Figure SI-3). U L<sub>III</sub>-edge EXAFS data of the sediment samples were also interpreted via shell-by-shell fitting of  $k^3$ weighted EXAFS spectra over 3-9.5 k using ARTEMIS<sup>29</sup>. The theoretical phase and amplitude functions in the fitting procedure were modeled using the following crystal structures: metatorbernite for U<sup>VI</sup>-O and U-P paths<sup>30</sup>, uraninite for U<sup>IV</sup>-O and U-U paths<sup>31</sup> and rutherfordine for U-C path <sup>32</sup>. The quality of the fits was estimated by the R-factor (Equation SI-1) and reduced  $X_v^2$  (Equation SI-2) parameters calculated by Artemis.

#### 2.3.7 Chemical extraction

Quantitative speciation of U in bioreduced RABS was performed via anoxic bicarbonate extraction according to a protocol based on Alessi et *al.*<sup>7</sup>. The chemical extraction was conducted in an anoxic atmosphere (3%:97% H<sub>2</sub>:N<sub>2</sub>) in a glove box (Coy Laboratory Products Inc, USA). Bioreduced RABS were resuspended in anoxic 50 mM sodium bicarbonate to extract the U(VI) fraction that was unreacted and adsorbed. Noncrystalline and mineral-adsorbed U(IV) was extracted by an anoxic 1 M sodium bicarbonate solution at pH ~9. Lastly, the sediments were digested in aqua regia (3:1 mixture by volume of concentrated HCl and HNO<sub>3</sub>) to determine the amount of recalcitrant crystalline U(IV) fraction (corresponding to UO<sub>2</sub>). The extraction was repeated at least in triplicates so that the error of the assay is given as a standard deviation amongst the replicates. Aliquots of the digests were filtered through 0.22 µm PTFE filters (ThermoFisher, USA), diluted by an appropriate factor in 0.1 M nitric acid (HNO<sub>3</sub>) and analyzed by ICP-MS for total U extracted from the solids.

#### 2.3.8 Incubation of SRC sediments for the aging of bioreduced species of U(IV)

After the U(VI) bioreduction phase in column experiments, sediments harvested from the first layer of SRC column 2 were incubated in static microcosms to investigate the impact of aging on the mineralogical structure of U(IV) under anoxic conditions. The experiment consisted of a series of eight serum bottles that were prepared under anoxic conditions in an anaerobic glove box with an atmospheric composition of 100% N<sub>2</sub> (MBraun, Germany). Each bottle contained 4 g of dried, homogenized sediment in 30 mL of anoxic RAGW (that includes 1 mM NaHCO<sub>3</sub>). Four of the bottles received RAGW amended with 10 mM (instead of 1 mM) bicarbonate. Every 4 months, one bottle per condition (high and low bicarbonate) was

sampled, the sediments were harvested by centrifugation, dried, ground and prepared for U XAS analysis at BL 11-2 at SSRL or BL I20-scanning at DLS as previously described.

## 2.4 Results and discussion

## 2.4.1 Bioreduction of U(VI) under sulfate-reducing conditions

During the initial 97 days of operation of the SRC columns, RABS gradually shifted from oxic to anoxic conditions as microbial processes were sustained by RAGW amended with electron donors but no U. However, U was observed in the effluent (Figure 2.1) because it occurs in RABS and is released through desorption and dissolution prior to the establishment of reducing conditions. XRF measurements of the original sediments revealed that RABS contained ~5 ppm of U. The total amount of U released from SRC columns was calculated by the trapezoidal integration method from the concentration of U in the effluent (Table SI-2). U released in the first 97 days varies between 1.3 and 1.8 mg, and this accounts for between 52 and 71% of the total content of U in RABS (Table SI-3).

After 20 days of operation, a few black spots appeared at the bottom of the columns and started extending through preferential flow paths in the sediment. The progressive color change was interpreted as evidence for the precipitation of iron sulfides as by-products of the metabolism of sulfate-reducing bacteria. After 120 days, the columns were entirely black. After day 121, as the U concentration in the effluent ranged between 0.3  $\mu$ M and not detectable (Figure 1), it was inferred that SRC columns achieved 100% removal of U from RAGW.

The majority of Fe in the effluent was Fe(II) (Figure SI-4 and Figure SI-5), consistent with the establishment of reducing conditions. Aqueous Fe(II) production occurred until day 106 as in previous columns experiments<sup>22,24,33</sup>. The concentration of sulfate in the effluents (SO<sub>4</sub><sup>2-</sup>) rapidly decreased after day 72 (Figure SI-6), suggesting sulfate reduction to sulfide with similar timing as in the previous work<sup>24</sup>. The decrease in

effluent concentrations of both Fe(II) and sulfate support the assumption that biogenic precipitation of FeS within the sediments is ongoing<sup>24,34</sup>.



Figure 2.1 Uranium concentration over time in the effluents of SRC columns 2, 3 and 4 (SRC2, SRC3, SRC4). Vertical lines indicate time periods of U amendment with the associated influent U concentration.

### 2.4.2 Chemical characterization of bioreduced sediments

Probing Fe content per column layer revealed that the concentration of Fe in the sediment does not differ significantly from the initial content in RABS (Figure SI-7), indicating that little Fe(II) is lost from the sediments, presumably due to FeS precipitation. Indeed, >90% of S is found as FeS in the sediment at the end of the experiment, based on S XANES (Figure SI-8, Table SI-4), and S was accumulated throughout the length of the column (Figure SI-9), with a significant enrichment relative to RABS. The first two column layers reach ~3 wt. % (2.7 wt. % for SRC3 and SRC4 and 3.2 wt. % for SRC2) relative to 0.05 wt. % for RABS (Figure SI-9).

LCF analysis of the iron (Fe) K edge EXAFS spectra (Figure SI-10) of RABS shows that iron was found predominantly as iron oxides (53%) (goethite, hematite) and clay minerals (45%) in the original sediments (Table SI-5), which is in agreement with the previous characterization by Mössbauer and XRD analysis reporting clay minerals, goethite, hematite, and magnetite<sup>33</sup>. Furthermore, the pool of Fe(III) in RABS is known to be readily bioavailable and capable of sustaining iron-reducing conditions for an extended period of time<sup>22,33</sup>. The ferric oxide content, which in the original RABS accounts for 53% of total Fe, is consumed in SRC where the only ferric oxide remaining after 407 days of in-column biostimulation was a small amount of goethite (4%) (Table SI-4). Therefore, our findings show that ferric oxides (including hematite) are extensively consumed. When RABS are biostimulated in the absence of sulfate33, hematite is persistent as a poorly bioavailable form of Fe(III) compared to other iron oxides such as ferrihydrite and goethite<sup>35</sup>. Therefore, we infer that, under sulfate-reducing condition, biogenic H2S is responsible for the extensive reduction of hematite. Consistently with the S XANES results (Table SI-5), mackinawite formed, representing 25% of the total Fe (as opposed to representing 2% in RABS). When the EXAFS of RABS is compared to the spectrum from the SRC, there is a distinctive feature at 8 Å-1<sup>7</sup> (Figure SI-10). The same feature is also found by Noel et al.<sup>36</sup> in sediments from sulfidic environments in Rifle, and it was attributed to the presence of iron sulfides after confirmation with Mössbauer analysis.

#### 2.4.3 Uranium speciation in the initial and aged samples

As observed in previous studies<sup>7,8</sup>, XRF analysis revealed that U was primarily immobilized in the bottom layer, closest to the inlet (Figure SI-11). Depending on the column, the content of U found in the first layer represented 61 to 76% of the total U that was immobilized in the entire SRC column. Therefore, as in previous column studies using RABS<sup>12,33,34</sup>, the columns' capacity to sequester U was not yet exhausted even after 407 days of flow-through. We attribute the high concentration of U in the inlet to the prereduction phase of 97 days that occurred before U was added to the influent. This allowed the establishment of strong reducing conditions and the reduction of U(VI) as it entered the column.

U LIII-edge XANES spectra of the sediments after bioreduction (SRC2 and SRC4) are shown in Figure SI-12 and analyzed by LCF. The model compounds used for LCF includes U(VI) as uranyl adsorbed onto ferrihydrite and U(IV) as non-crystalline tetravalent uranium (NCU4) that was synthesized via U(VI) bioreduction by Shewanella oneidensis MR-1 (Figure SI-1). LCF revealed that the sediments post-reduction contained predominantly tetravalent uranium species although SRC2 included ~14% U(VI). A chemical extraction confirmed that NCU4 is the dominant species. In fact, the assay indicated that NCU4 accounted for 70-75% of the total U while U(VI) accounted for 17-21%. The acid digestion reports that UO<sub>2</sub> content varies between 8 and 11 % (Table SI-7).

The Fourier transforms of the U L<sub>III</sub>-edge EXAFS spectra for SRC2 and SRC4 are presented in Figure SI-13. The parameters obtained by the shell-by-shell fitting procedure are reported in Table. 2.1 and Figure 2.2. As predicted by the XANES LCF, the fit is characterized by a minor contribution from U-O path (U-O<sub>ax</sub>) at ~1.76  $\pm$  0.02 Å which is consistent with U-O axial in uranyl and accounts for unreacted U(VI) fraction (Table 2.1). The Fourier transforms showed a dominant frequency at ~1.8 Å (r+ $\Delta$ r) which is typical of oxygen coordination of U. This feature was fitted by two subshells at 2.37 and 2.46 Å as in previous studies where tetravalent and hexavalent uranium co-exist<sup>13,37</sup>. The first is typical of U-O in U(IV), the latter represents equatorial U-O (U-O<sub>eq</sub>) in uranyl. In order to reduce the number of fit variables, the coordination numbers of the three U-O (U-O<sub>ax</sub>, U-O<sub>eq</sub>, and U-O) shells were linked assuming a total of 8 O neighbors. Each model contained three multi-scatter paths for the U-O coordination. The variables for the multiscatter paths are defined as equal to the variables of the single scatter for U-O, thus the addition of multiscatter paths improved the fit without increasing the reduced Chi squared parameter (X<sub>r</sub><sup>2</sup>).

The fits were further improved by including 2±0.8 U-C pairs at a distance of 2.93 Å, which is compatible with bidentate mononuclear complexes and  $3.7\pm0.6$  P at 3.59 Å which is compatible with a monodentate bond as presented in the models of U(IV) complexed with carboxyl groups<sup>8</sup> and phosphoryl groups<sup>7,8</sup> and

as previously found in bioreduced NCU4 in natural sediments<sup>2,13,18,38</sup> and in microbially reduced U(IV)<sup>5,7,8</sup>. Our model is representative of the experimental data up to ~3.5 Å, and the peak at 3.6 Å (r+ $\Delta$ r) corresponding to U-U pair correlations in the Fourier transform could not be found. Therefore, we conclude that the occurrence of uraninite <sup>2,3,12,18</sup> is below the detection limit of bulk U L<sub>III</sub>-edge EXAFS.

				1 mM HCO3						10 mM HCO3					
shell		initial (SRC2)		4 months		8 months		12 months		4 months		8 months		12 months	
U-O <sub>ax</sub>	CN	0.6	(10)	0.5	(1)	0.4	0.1	0.3	(1)	0.2	(1)	0.3	(1)	0.3	(1)
	R	1.76	(2)	1.73	(5)	1.71	(8)	1.69	(3)	1.69	(7)	1.72	(4)	1.70	(3)
	σ²	0.003	-	0.006	(3)	0.003	(2)	0.003		0.003	-	0.003	-	0.003	-
	E <sub>0</sub>	6.4	(3)	4.7	(29)	2.8		3.9	(16)	4.5	(19)	4.0	(23)	4.2	(23)
U-O <sub>eq</sub>	CN	1.8	**	1.5	**	1.2	**	0.9	**	0.7	**	0.9	**	0.9	**
	R	2.46	*	2.49	*	2.44	*	2.39	*	2.39	*	2.44	*	2.40	*
	σ²		*		*		*		*		*		*		*
	Eo		*		*		*		*		*		*		*
U-C	CN	2.0	(8)	6.1	(3)	4.3	(13)	5.2	(10)	4.8	(15)	4.9	(16)	4.9	(11)
	R	2.93	-	2.93	-	2.93	-	2.93	-	2.93	-	2.93	-	2.93	-
	σ²		*	0.006	*		*		*		*		*		*
	E <sub>0</sub>		*	4.7	*	3.9	(12)		*		*		*		*
U-0	CN	5.9	**	6.0	**	6.4	**	6.8	**	7.1	**	6.8	**	6.8	**
	R	2.37	(1)	2.33	(4)	2.38	(6)	2.32	(2)	2.32	(3)	2.33	(2)	2.32	(2)
	σ²	0.009	(1)	0.012	(2)	0.009	(1)	0.013	(1)	0.012	(2)	0.012	(2)	0.013	(1)
	E <sub>0</sub>	-0.2	(14)	-2.6	(58)	1.8	(22)	-2.5	(29)	-0.4	(37)	0.6	(30)	-1.9	(38)
U-P	CN	3.7	(6)	4.6	(7)	4.2	(14)	3.6	(9)	4.8	-	4.8	-	4.8	(52)
	R	3.59	-	3.59	-	3.59	-	3.59	-	3.62	(2)	3.63	(6)	3.59	-
	σ²	0.008	(2)	0.009	(2)	0.009	(2)	0.008	(1)	0.012	(4)	0.011	(3)	0.012	(1)
	Eo	0.3	(28)	1.4	(33)	1.7	(25)	1.8	(20)	1.6	(18)	1.1	(23)	1.4	(31)
R-factor		0.009		0.015		0.023		0.017		0.012		0.019		0.012	

Table 2.1 Results of shell-by-shell fitting procedure of U LIII edge EXAFS spectra of SRC 2 after bioreduction phase (no aging) and after an aging period of 4, 8 and 12 months at 1 and 10 mM HCO3 RAGW.

Notes: EXAFS fitting parameters include coordination number (CN), interatomic distances (R(Å)), Debye-Waller factor ( $\sigma^2(Å)$ ) and energy shift  $\Delta E_0$  (eV). The uncertainties of the fit are given in parenthesis for the last significant figure. (\*) parameters linked to the one above in the table. (-) fixed parameter. (\*\*) the total number of oxygen atoms is constrained to 8. Each model contains one MS path for the uranyl moiety. The accuracy of the fit is evaluated by  $R_f$  as defined in equation SI-1.

Thus, the results of XANES LCF, chemical extraction, and EXAFS shell-by-shell fits are consistent and reveal that U found in the first layer is present predominantly as non-crystalline U(IV) that is complexed with C and P.

Sediments from SRC2 were aged for 4, 8 and 12 months in the presence of either 1 mM or 10 mM HCO<sub>3</sub><sup>-</sup>. U EXAFS in aged sediments was fit following the same procedure applied for fresh SRC2. Figure 2a and Figure 2d show the U L<sub>III</sub>-edge XANES spectra and LCFs for the initial sediment before aging and the aged sediments at low bicarbonate concentration and high bicarbonate concentration. The EXAFS spectra and the shell by shell fits are shown in K- (Figure 2.2b and Figure 2.2e) and R space (Figure 2.2c and Figure 2.2f). The results of the LCF are reported in Table 2.1.

The aged samples are characterized by the same dominant features observed in the initial sample. Even though the contribution of U-O<sub>ax</sub> is small, we observe a progressive decrease of CN over the duration of the incubation from 0.6  $\pm$ 0.1 in the initial sediment to 0.3 $\pm$ 0.1 at 12 months in samples that are aged in the presence of low bicarbonate (1 mM) suggesting that the pool of U(VI) is gradually consumed and becomes undetectable after 12 months of incubation. It is possible that, in the absence of exogenous electron donor, the reduction of U(VI) was mediated by FeS or Fe(II)-bearing solids. In contrast, when sediments are incubated in the higher bicarbonate concentration (10 mM), the CN of U-O<sub>ax</sub> ranges between 0.2 $\pm$ 0.1 and 0.3 $\pm$ 0.1, suggesting that high bicarbonate concentration desorb unreacted U(VI) after the formation of soluble U(VI)-carbonates complexes.

Thus, based on this analysis, there is no evidence for the formation of UO<sub>2</sub> through aging of NCU4. In order to carry out a sensitivity analysis and determine whether the shell-by-shell fits could include a small contribution from a U-U pair correlation at 3.8 Å, which would be characteristic of UO<sub>2</sub>, we included the single scattering path from U-U in uraninite in the model of 12 months aged samples at low and high bicarbonate concentration. In this analysis,  $\Delta r$ ,  $\sigma^2$ , and E<sub>0</sub> in U-U were set equal to those in U-O as the theoretical amplitude function of U-U was calculated in FEFF using the same reference as was used for the U-O path<sup>31</sup>. Therefore, CN was the only additional floating variable in the new models including U-U. Despite the decreased values of  $R_f$ , the goodness of the fits was not improved as the addition of one extra variable caused an increase in  $X_R^2$  both at low and high bicarbonate loading (Table SI-8, Tables-15).

Scanning transmission electron microscopy (STEM) revealed that, due to the heterogeneity in natural sediments, U occurs distributed on the surface of FeS grains (Figures SI-17 and SI-18) as well as single high-concentration spots (Figure SI-19), which could correspond to a mineral precipitate such as UO<sub>2</sub>.

Despite evidence for the presence of some U hotspots, U L<sub>III</sub> bulk EXAFS indicate that U speciation does not change after 12 months and that NCU4 is a stable phase under these conditions. While this is in contrast with previous observation of UO<sub>2</sub> forming from NCU4 after 15 months of incubation<sup>18</sup>, our work is in substantial agreement with the persistence of NCU4 in the environment in pristine organic-rich environments<sup>14</sup>, in wetland<sup>39</sup> and in biostimulated sediments that were aged *in-situ* for 1 year<sup>2</sup>.



Figure 2.2 U LIII edge XANES (left panels), shell-by-shell fits of the unfiltered EXAFS (center panels) and their Fourier Transforms (right) for fresh U after bioreduction in a column experiment and sediments that were aged for 4, 8 and 12 months at low bicarbonate concentration (bottom panels) and high bicarbonate concentration (top panels). Experimental data are as solid black and model fits as red dashed lines. Data and fits in panels a, b and c are from sediments aged 4, 8 and 12 months at 1 mM HCO3. Data and fits in panels d, c and e are from sediments aged 4, 8, 12 months at 10 mM HCO3. The fitting parameters are reported in table 1.

## 2.5 Environmental Implications

This work shows that NCU4 speciation does not change over a period of 12 months under anoxic conditions. This is consistent with findings from environments in which NCU4 was identified: a contaminated wetland in France harbored NCU4 that had been formed several decades ago<sup>4</sup>, an alpine meadow<sup>13,16</sup>, an organic-rich roll front deposit<sup>14</sup>. This also means that bioremediation of U in the subsurface, which has been shown to produce NCU4<sup>5</sup>, would likely result in the persistence of NCU4 if anoxic conditions are maintained. With regards to the effectiveness of bioreduction as bioremediation strategy, this is an undesirable result, because transformation to the more stable UO<sub>2</sub> would be preferable. Thus, the persistence of NCU4 would render a remediated site susceptible to U remobilization through events (i.e., change of the water table level) that bring oxygen to the subsurface.

## 2.6 Acknowledgments

This study is funded by the Swiss National Science Foundation under Grant # 200020-144335. We thank Jean-Claude Lavanchy from the University of Lausanne for assistance during XRF analysis. The U L<sub>III</sub>-edge XAS, S K-edge and Fe K-edge XAS experiments were performed on the 11-2,4-3 and 4-1 beamline at the Stanford Synchrotron Radiation Lightsource (SSRL). We acknowledge for technical assistance received during the XAS analyses from Ryan Davis, Erik Nelson, and Matthew Latimer. Use of the SSRL, SLAC National Accelerator Laboratory is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract DE-AC02-76SF00515. We acknowledge Vincent Noel for sharing Mackinawite model compound which significantly improved the XAS analysis of Fe and S data. We acknowledge Shusaku Hayama at the Diamond Light Source (Beamline I20-scanning), for assistance with the U L<sub>III</sub>-edge EXAFS spectroscopy measurements (project SP17472).

## 2.7 References

(1) Lovley, D. R.; Phillips, E. J. Reduction of Uranium by Desulfovibrio Desulfuricans. *Appl. Environ. Microbiol.* **1992**, *58* (3), 850–856.

(2) Bargar, J. R.; Williams, K. H.; Campbell, K. M.; Long, P. E.; Stubbs, J. E.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Alessi, D. S.; Stylo, M.; Webb, S. M.; et al. Uranium Redox Transition Pathways in Acetate-Amended Sediments. *PNAS* **2013**, *110* (12), 4506–4511. https://doi.org/10.1073/pnas.1219198110.

(3) Alessi, D. S.; Lezama-Pacheco, J. S.; Janot, N.; Suvorova, E. I.; Cerrato, J. M.; Giammar, D. E.; Davis, J. A.; Fox, P. M.; Williams, K. H.; Long, P. E.; et al. Speciation and Reactivity of Uranium Products Formed during in Situ Bioremediation in a Shallow Alluvial Aquifer. *Environ. Sci. Technol.* **2014**, *48* (21), 12842–12850. https://doi.org/10.1021/es502701u.

(4) Wang, Y.; Frutschi, M.; Suvorova, E.; Phrommavanh, V.; Descostes, M.; Osman, A. A. A.; Geipel, G.; Bernier-Latmani, R. Mobile Uranium(IV)-Bearing Colloids in a Mining-Impacted Wetland. *Nature Communications* **2013**, *4*, 2942. https://doi.org/10.1038/ncomms3942.

(5) 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. Non-Uraninite Products of Microbial U(VI) Reduction. *Environ. Sci. Technol.* 2010, 44 (24), 9456–9462. https://doi.org/10.1021/es101675a.

(6) Stylo, M.; Neubert, N.; Roebbert, Y.; Weyer, S.; Bernier-Latmani, R. Mechanism of Uranium Reduction and Immobilization in Desulfovibrio Vulgaris Biofilms. *Environ. Sci. Technol.* **2015**, *49* (17), 10553– 10561. https://doi.org/10.1021/acs.est.5b01769.

(7) Alessi, D. S.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Janousch, M.; Bargar, J. R.; Persson, P.; Bernier-Latmani, R. The Product of Microbial Uranium Reduction Includes Multiple Species with U(IV)–Phosphate Coordination. *Geochimica et Cosmochimica Acta* 2014, 131, 115–127. https://doi.org/10.1016/j.gca.2014.01.005.

Boyanov, M. I.; Fletcher, K. E.; Kwon, M. J.; Rui, X.; O'Loughlin, E. J.; Löffler, F. E.; Kemner, K. M.
 Solution and Microbial Controls on the Formation of Reduced U(IV) Species. *Environ. Sci. Technol.* 2011, 45 (19), 8336–8344. https://doi.org/10.1021/es2014049.

(9) Cerrato, J. M.; Ashner, M. N.; Alessi, D. S.; Lezama-Pacheco, J. S.; Bernier-Latmani, R.; Bargar, J. R.;
 Giammar, D. E. Relative Reactivity of Biogenic and Chemogenic Uraninite and Biogenic Noncrystalline
 U(IV). *Environ. Sci. Technol.* 2013, 47 (17), 9756–9763. https://doi.org/10.1021/es401663t.

Anderson, R. T.; Vrionis, H. A.; Ortiz-Bernad, I.; Resch, C. T.; Long, P. E.; Dayvault, R.; Karp, K.;
 Marutzky, S.; Metzler, D. R.; Peacock, A.; et al. Stimulating the In Situ Activity of Geobacter Species To
 Remove Uranium from the Groundwater of a Uranium-Contaminated Aquifer. *Appl. Environ. Microbiol.* 2003, 69 (10), 5884–5891. https://doi.org/10.1128/AEM.69.10.5884-5891.2003.

Ulrich, K.-U.; Singh, A.; Schofield, E. J.; Bargar, J. R.; Veeramani, H.; Sharp, J. O.; Bernier-Latmani,
 R.; Giammar, D. E. Dissolution of Biogenic and Synthetic UO2 under Varied Reducing Conditions. *Environ. Sci. Technol.* 2008, 42 (15), 5600–5606. https://doi.org/10.1021/es800647u.

(12) Sharp, J. O.; Lezama-Pacheco, J. S.; Schofield, E. J.; Junier, P.; Ulrich, K.-U.; Chinni, S.; Veeramani, H.; Margot-Roquier, C.; Webb, S. M.; Tebo, B. M.; et al. Uranium Speciation and Stability after Reductive Immobilization in Aquifer Sediments. *Geochimica et Cosmochimica Acta* **2011**, *75* (21), 6497–6510. https://doi.org/10.1016/j.gca.2011.08.022.

(13) Mikutta, C.; Langner, P.; Bargar, J. R.; Kretzschmar, R. Tetra- and Hexavalent Uranium Forms Bidentate-Mononuclear Complexes with Particulate Organic Matter in a Naturally Uranium-Enriched Peatland. *Environmental Science & Technology* **2016**, *50* (19), 10465–10475. https://doi.org/10.1021/acs.est.6b03688.

Bhattacharyya, A.; Campbell, K. M.; Kelly, S. D.; Roebbert, Y.; Weyer, S.; Bernier-Latmani, R.; Borch,
 T. Biogenic Non-Crystalline U<sup>(IV)</sup> Revealed as Major Component in Uranium Ore Deposits. *Nature Communications* 2017, *8*, 15538. https://doi.org/10.1038/ncomms15538.

Noël, V.; Boye, K.; Lezama Pacheco, J. S.; Bone, S. E.; Janot, N.; Cardarelli, E.; Williams, K. H.; Bargar, J. R. Redox Controls over the Stability of U(IV) in Floodplains of the Upper Colorado River Basin. *Environ. Sci. Technol.* 2017, *51* (19), 10954–10964. https://doi.org/10.1021/acs.est.7b02203.

(16) Regenspurg, S.; Margot-Roquier, C.; Harfouche, M.; Froidevaux, P.; Steinmann, P.; Junier, P.; Bernier-Latmani, R. Speciation of Naturally-Accumulated Uranium in an Organic-Rich Soil of an Alpine Region (Switzerland). *Geochimica et Cosmochimica Acta* 2010, 74 (7), 2082–2098. https://doi.org/10.1016/j.gca.2010.01.007.

(17) Kelly, S. D.; Wu, W.-M.; Yang, F.; Criddle, C. S.; Marsh, T. L.; O'Loughlin, E. J.; Ravel, B.; Watson, D.;
Jardine, P. M.; Kemner, K. M. Uranium Transformations in Static Microcosms. *Environ. Sci. Technol.* 2010, 44 (1), 236–242. https://doi.org/10.1021/es902191s.

(18) Newsome, L.; Morris, K.; Shaw, S.; Trivedi, D.; Lloyd, J. R. The Stability of Microbially Reduced U(IV);
 Impact of Residual Electron Donor and Sediment Ageing. *Chemical Geology* 2015, *409* (Supplement C),
 125–135. https://doi.org/10.1016/j.chemgeo.2015.05.016.

(19) Stylo, M.; Alessi, D. S.; Shao, P. P.; Lezama-Pacheco, J. S.; Bargar, J. R.; Bernier-Latmani, R. Bioge-ochemical Controls on the Product of Microbial U(VI) Reduction. *Environmental Science & Technology* 2013, 47 (21), 12351–12358. https://doi.org/10.1021/es402631w.

Yabusaki, S. B.; Fang, Y.; Long, P. E.; Resch, C. T.; Peacock, A. D.; Komlos, J.; Jaffe, P. R.; Morrison,
S. J.; Dayvault, R. D.; White, D. C.; et al. Uranium Removal from Groundwater via in Situ Biostimulation:
Field-Scale Modeling of Transport and Biological Processes. *J. Contam. Hydrol.* 2007, *93* (1–4), 216–235.
https://doi.org/10.1016/j.jconhyd.2007.02.005.

(21) Moon, H. S.; Komlos, J.; Jaffé, P. R. Uranium Reoxidation in Previously Bioreduced Sediment by Dissolved Oxygen and Nitrate. *Environ. Sci. Technol.* **2007**, *41* (13), 4587–4592. https://doi.org/10.1021/es063063b.

(22) Campbell, K. M.; Kukkadapu, R. K.; Qafoku, N. P.; Peacock, A. D.; Lesher, E.; Williams, K. H.; Bargar, J. R.; Wilkins, M. J.; Figueroa, L.; Ranville, J.; et al. Geochemical, Mineralogical and Microbiological Characteristics of Sediment from a Naturally Reduced Zone in a Uranium-Contaminated Aquifer. *Applied Geochemistry* **2012**, *27* (8), 1499–1511. https://doi.org/10.1016/j.apgeochem.2012.04.013.

Moon, H. S.; McGuinness, L.; Kukkadapu, R. K.; Peacock, A. D.; Komlos, J.; Kerkhof, L. J.; Long, P.
 E.; Jaffé, P. R. Microbial Reduction of Uranium under Iron- and Sulfate-Reducing Conditions: Effect of Amended Goethite on Microbial Community Composition and Dynamics. *Water Research* 2010, *44* (14), 4015–4028. https://doi.org/10.1016/j.watres.2010.05.003.

(24) Mehta, V. S.; Maillot, F.; Wang, Z.; Catalano, J. G.; Giammar, D. E. Transport of U(VI) through Sediments Amended with Phosphate to Induce in Situ Uranium Immobilization. *Water Research* **2015**, *69* (Supplement C), 307–317. https://doi.org/10.1016/j.watres.2014.11.044.

(25) Campbell, K. M.; Davis, J. A.; Bargar, J.; Giammar, D.; Bernier-Latmani, R.; Kukkadapu, R.; Williams, K. H.; Veramani, H.; Ulrich, K.-U.; Stubbs, J.; et al. Composition, Stability, and Measurement of Reduced Uranium Phases for Groundwater Bioremediation at Old Rifle, CO. *Applied Geochemistry* **2011**, *26*, S167–S169. https://doi.org/10.1016/j.apgeochem.2011.03.094.

(26) Komlos John; Mishra Bhoopesh; Lanzirotti Antonio; Myneni Satish C. B.; Jaffé Peter R. Real–Time Speciation of Uranium during Active Bioremediation and U(IV) Reoxidation. *Journal of Environmental Engineering* **2008**, *134* (2), 78–86. https://doi.org/10.1061/(ASCE)0733-9372(2008)134:2(78).

(27) Stookey, L. L. Ferrozine---a New Spectrophotometric Reagent for Iron. *Anal. Chem.* **1970**, *42* (7), 779–781. https://doi.org/10.1021/ac60289a016.

(28) Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: Data Analysis for X-Ray Absorption Spectroscopy Using IFEFFIT. *J Synchrotron Radiat* **2005**, *12* (Pt 4), 537–541. https://doi.org/10.1107/S0909049505012719.

(29) Ross, M.; Evans, H. T.; Appleman, D. E. Studies of the Torbernite Minerals (II): The Crystal Structure of Meta-Torbernite. *American Mineralogist* **1964**, *49* (11–12), 1603–1621.

(30) AMCSD Search Results http://rruff.geo.arizona.edu/AMS/minerals/Uraninite (accessed Jul 31, 2018).

(31) FINCH, R. J.; ANDFRANK, M. A. C.; HAWTHORNE, C. REFINEMENOTFTHECRYSTASL TRUC-TUROEFRUTHERFORDINE. *THE CANADIAN MINERALOGIST* 10.

(32) Komlos, J.; Peacock, A.; Kukkadapu, R. K.; Jaffé, P. R. Long-Term Dynamics of Uranium Reduction/Reoxidation under Low Sulfate Conditions. *Geochimica et Cosmochimica Acta* **2008**, *72* (15), 3603– 3615. https://doi.org/10.1016/j.gca.2008.05.040.

Moon, H. S.; Komlos, J.; Jaffé, P. R. Biogenic U(IV) Oxidation by Dissolved Oxygen and Nitrate in Sediment after Prolonged U(VI)/Fe(III)/SO42– Reduction. *Journal of Contaminant Hydrology* 2009, *105* (1), 18–27. https://doi.org/10.1016/j.jconhyd.2008.10.014.

(34) Roden, E. E.; Zachara, J. M. Microbial Reduction of Crystalline Iron(III) Oxides: Influence of Oxide Surface Area and Potential for Cell Growth. *Environmental Science & Technology* **1996**, *30* (5), 1618–1628. https://doi.org/10.1021/es9506216.

(35) Noël, V.; Boye, K.; Kukkadapu, R. K.; Bone, S.; Lezama Pacheco, J. S.; Cardarelli, E.; Janot, N.; Fendorf, S.; Williams, K. H.; Bargar, J. R. Understanding Controls on Redox Processes in Floodplain Sediments of the Upper Colorado River Basin. *Science of The Total Environment* **2017**, *603–604*, 663–675. https://doi.org/10.1016/j.scitotenv.2017.01.109.

(36) Kelly, S. D.; Kemner, K. M.; Carley, J.; Criddle, C.; Jardine, P. M.; Marsh, T. L.; Phillips, D.; Watson,
D.; Wu, W.-M. Speciation of Uranium in Sediments before and after In Situ Biostimulation. *Environ. Sci. Technol.* 2008, 42 (5), 1558–1564. https://doi.org/10.1021/es071764i.

(37) Stetten, L.; Mangeret, A.; Brest, J.; Seder-Colomina, M.; Le Pape, P.; Ikogou, M.; Zeyen, N.; Thouvenot, A.; Julien, A.; Alcalde, G.; et al. Geochemical Control on the Reduction of U(VI) to Mononuclear U(IV) Species in Lacustrine Sediments. *Geochimica et Cosmochimica Acta* **2018**, *222* (Supplement C), 171–186. https://doi.org/10.1016/j.gca.2017.10.026.

Wang, Y.; Bagnoud, A.; Suvorova, E.; McGivney, E.; Chesaux, L.; Phrommavanh, V.; Descostes, M.;
 Bernier-Latmani, R. Geochemical Control on Uranium(IV) Mobility in a Mining-Impacted Wetland. *Environ. Sci. Technol.* 2014, 48 (17), 10062–10070. https://doi.org/10.1021/es501556d.

# Chapter 3 The role of iron sulfide phases in the stability of non-crystalline tetravalent uranium in natural sediments

This chapter explores the role of mackinawite in the stability of non-crystalline tetravalent uranium in the sediments previously described in chapter 2. We present the results of a series of oxidation experiments under varying conditions in batch and flow-through systems where we monitor oxidation and U release over time in the presence and absence of FeS. We introduce an oxidative mechanism where FeS, under certain conditions (i.e., high dissolved oxygen concentration), indirectly contributes to the oxidation and remobilization of U via the production of reactive oxygen species.

This chapter is presented as a manuscript to be submitted for publication in Environmental Science and technology.

Supporting information of this manuscript are presented in Annex 2.

*Luca Loreggian*; experimental work and associated wet chemistry (ICP-MS, ICP-OES, IC) and mineralogical (XAS) analysis. *Rizlan Bernier-Latmani*; conceptual guidance and extensive manuscript review.

## 3.1 Abstract

Uranium (U) in-situ bioremediation has been largely investigated as a cost-effective strategy to tackle U contamination in the subsurface. Uraninite was initially considered the only product of reduction, but numerous studies revealed that non-crystalline U(IV) (NCU4) species prevail after bioreduction. This new finding questions the effectiveness of bioremediation because NCU4 species are labile and susceptible to oxidation. In this regard, understanding the stability of NCU4 in the environment is crucial. In particular, the impact of Fe(II)-minerals (such as FeS) associated with U(IV) in sediments on the stability of NCU4 has been overlooked. A previous study revealed that FeS accelerates the oxidation of U(IV), but the exact pathway remained to be identified. In this work, we show that reactive oxygen species (ROS), formed via the oxidation of FeS, partially contribute to the oxidation of NCU4. ROS are environmentally relevant oxidants that are detected in the field at the oxic-anoxic interface where oxygen meets reduced species. Whilst ROS are known to oxidize iron, copper, manganese, and arsenic, their impact on U has been hypothesized but never demonstrated before. This work provides evidence that ROS, produced after the oxidation of Fe(II)-species, contribute to the rapid oxidation and release of NCU4 into solution.

*Keywords:* amorphous tetravalent uranium, oxidation, reactive oxygen species, uraninite, mackinawite, Xray absorption spectroscopy

## 3.2 Introduction

Uranium (U) contamination in the subsurface is a concern in former and present uranium mining, milling, and processing sites. When dissolved U is found at elevated concentrations in aquifers, health risks and regulatory bodies may impose remediation. U mobility depends on speciation and redox state, with reducing species being in general relatively insoluble and immobile. Thus, reduction of U, either through biological activity<sup>3</sup> or abiotic processes<sup>4</sup>, results in the net immobilization of U. Uranium bioremediation through the stimulation of the native microbial community to reduce U(VI) has been considered a convenient, cost-effective option<sup>5</sup> to more invasive remediation options such as excavation.

On the other hand, while uraninite (UO<sub>2</sub>) was initially believed to be the dominant species in bioreduced U(IV), laboratory and field studies have revealed that non-crystalline tetravalent species (NCU4) prevail after biotic reduction<sup>6–8</sup> and in naturally reduced zones<sup>9,10</sup>. NCU4 appears to be more sensitive than UO<sub>2</sub> to oxidation by oxygen<sup>11–13</sup> and to form soluble carbonate complexes more readily than UO<sub>2</sub><sup>14</sup>. As the success of bioremediation depends on the resistance of U(IV) to reoxidation and release to solution, understanding NCU4 stability in the environment is crucial to assess the effectiveness of bioremediation in the long term. In particular, little is known about the effect of reduced species, such as iron-sulfide and other Fe(II)-bearing minerals often found in association with U in the environment, on the oxidation of U(IV) by oxidants such as O<sub>2</sub>. For example, mackinawite (FeS) is a typical product of the metabolism of sulfate-reducing bacteria in bioreduced zones<sup>7,15</sup>. In laboratory settings, FeS has been shown to either protect<sup>13</sup> or enhance the oxidative dissolution of U(IV)<sup>12</sup>, depending on the speciation of U(IV). However, its role remains ill-defined in environmentally-relevant conditions.

Recently, the dark production of reactive oxygen species (ROS) has been demonstrated in laboratory studies<sup>14,15</sup> and in field investigations<sup>16,17</sup>. ROS production is more likely to occur at oxic-anoxic interfaces where reduced species meet oxygen<sup>16</sup>. Indeed, it has been reported that Fe(II) species in the subsurface are involved in the dark production of ROS via Fenton's reaction <sup>1,2,16,18,19</sup>. A significant amount of ROS was reported during the oxidation of reduced sediments containing phyllosilicates<sup>14</sup>, iron sulfides<sup>15</sup> or pyrite<sup>20</sup>. As ROS are highly reactive molecules with low-selectivity<sup>17</sup>, they act as oxidants with iron<sup>18,21</sup>, copper<sup>22</sup>, manganese<sup>23</sup>, and arsenic<sup>1,2</sup>. It has even been proposed that ROS formation at the oxic/anoxic interfaces may play a role in controlling redox speciation of uranium<sup>15,16</sup>.

This work investigates the hypothesis that ROS affect U geochemistry and contribute to NCU4 oxidation and release into solution. More specifically, we hypothesize that the oxygen-mediated oxidation of Fe(II) species drives ROS production, which in turn, drives oxidation and release of NCU4 into solution. Because ROS production is linked to total Fe(II) content and Fe speciation, these two variables will likely affect the rate and the extent of oxidation indirectly.

The aims of this work are: (i) to investigate the stability of sediment U(IV) during exposure to  $O_2$ , (ii) to determine the impact of FeS on the resistance of NCU4 to oxidation, and (iii) to uncover whether ROS production contributes to NCU4 oxidation.

Sediments from the Old Rifle site (CO, USA) were biostimulated with anoxic artificial groundwater amended with uranium and multiple electron donors under (i) sulfate-reducing conditions to favor the formation of FeS and (ii) iron-reducing conditions as a control system lacking FeS. The stability of NCU4 was tested under different geochemical conditions in batch incubation and in continuously-stirred reactors with a steady flow of oxic or suboxic groundwater to mimic the aquifer in Rifle.

## 3.3 Materials and methods

#### 3.3.1 Sediments

The sediments (Rifle Area Background Sediments, RABS) used in this study were collected in the background area of a former U mining and milling processing site in Rifle, (CO, USA). RABS were biostimulated
in columns experiments with artificial groundwater amended with uranyl acetate to favor the precipitation of non-crystalline tetravalent uranium (NCU4) (manuscript in preparation). Two distinct influents were used for these columns. Artificial groundwater with a composition representative of the site groundwater (Rifle Artificial Groundwater, RAGW) (Table SI-1) that includes sulfate (14 mM) and that yielded U(IV) formation under sulfate-reducing conditions (SRC). The results of this experiment including a detailed characterization of the NCU4 produced within the sediments were presented in an earlier publication (manuscript in preparation). The second condition utilized the same RAGW except that it excluded sulfate. Hence, the conditions established within the column were iron-reducing conditions (IRC) and the effluent composition as well as U and Fe speciation in the sediments are presented in this work (the IRC column is referred to as IRC6). In this case, bioavailable Fe(III), which naturally occurs in RABS<sup>24</sup>, was the primary electron acceptor. Thus, the IRC sediments lacked iron sulfides and served as a control system to investigate the role of FeS during oxidation of NCU4 under conditions that mimic the subsurface environment (characterization of IRC sediments is presented in SI).

#### 3.3.2 Batch oxidation experiments

Batch experiments were performed with 3g/L of SRC and IRC sediments with oxic and suboxic RAGW in glass bottles that were hermetically closed with a butyl rubber stopper and a crimper. The bottles had a total volume of 240 mL and the suspension represented a volume of 30 mL. Experiments were run in triplicate, but entire bottles were sacrificed at specific time points (3, 6, and 15 hours). Prior to use in these experiments, SRC and IRC sediments were washed with anoxic 50 mM HCO<sub>3</sub> to remove unreacted U(VI) adsorbed onto the solids. RAGW contained dissolved oxygen (DO) at a concentration of 8.56 mg/L for experiments under oxic conditions, and 2.14 mg/L for experiments under suboxic conditions. The suspensions of RAGW and sediments were continuously shaken in the dark and the supernatant routinely sampled and filtered through 0.22 µm PTFE filter (ThermoFisher, USA) for quantification of dissolved U

and S. In order to assess the contribution of ROS to U(IV) oxidation, ROS production was quenched by the addition of 50 ku/L of superoxide dismutase (SOD) and 100 ku/L of catalase (CAT) (Sigma Aldrich, USA) in control experiments. To counter CAT unstability and degradation, 150  $\mu$ L aliquots of CAT stock solution (200kU/L) was added to RAGW every 2 hours.

At the end of the experiment, solid samples were collected for U speciation by chemical extraction and for S speciation by X-ray absorption spectroscopy. Additional solid samples were obtained for U speciation after 3 and 6 hours of oxidation.

# 3.3.3 Flow-through oxidation experiments

Oxic and suboxic flow-through oxidation experiments were conducted in 12.5 mL Plexiglas continuously stirred tank reactors (CSTRs) with an influent composed of RAGW containing either 1 mM or 10 mM bicarbonate concentration and no gas phase. Oxic experiments were run in triplicate but entire reactors were sacrificed at specific time points (21, 68, and 296 hours). Suboxic experiments were run in duplicate. The influent was stored in a Tedlar bag to avoid gas exchange with the atmosphere and to maintain constant pH, DO and bicarbonate concentration for the duration of the experiments. The suboxic experiments were conducted inside an anaerobic chamber (Coy Laboratory Products Inc, USA) with an atmosphere of 3%:97% H<sub>2</sub>: N<sub>2</sub>. Each reactor was loaded with 1 g of SRC or IRC sediments. The flow rate was maintained between 0.9 and 1.1 mL/h by a peristaltic pump (Ismatec IP, Switzerland) and gravimetrically monitored during sampling. The resulting hydraulic residence time was ~12.5 hours. Effluent samples from the CSTRs were filtered through 0.22 µm PTFE filter (ThermoFisher, USA) at the outlet of the reactors. At the end of the experiment, solid samples were collected for U speciation by chemical extraction, and Fe and S speciation by X-ray absorption spectroscopy. Additional solid samples were collected for time series speciation in the oxic case after 21 and 68 hours of oxidation.

# 3.3.4 Analyses

Filtered samples from the flow-through and batch experiments were diluted and preserved in 0.1 M HNO<sub>3</sub> at 4 °C until analysis. Total dissolved uranium and sulfur were determined respectively by inductivelycoupled plasma mass spectrometry (ICP-MS; Perkin Elmer ELAN DRC II) and Inductively-coupled plasma optical emission spectrometry (ICP-OES; Multitype ICP Emission Spectrometer, ICPE-9000, Shimadzu). The content and speciation of U remaining in the solids after oxidation were characterized by chemical extraction on dried and homogenized sediments in the anoxic atmosphere  $(3\%:97\% H_2:N_2)$  of a glove box (Cov Laboratory Products Inc, USA) according to a protocol based on Alessi et al.<sup>12</sup>. Sediments were split into three aliquots. One aliquot was resuspended in anoxic 50 mM sodium bicarbonate to extract the U(VI) fraction that was unreacted and adsorbed. The second was used to extract U(VI) as well as non-crystalline and mineral adsorbed U(IV) using an anoxic 1 M sodium bicarbonate solution at pH ~9. The contributino of NCU4 was then calculated by subtraction of the amount of U released by 50 mM NaHCO<sub>3</sub> extraction from that released by the 1M NaHCO<sub>3</sub> extraction. The third aliquot was digested in aqua regia (3:1 mixture by volume of concentrated HCl and HNO<sub>3</sub>) to determine the total amount of U. The recalcitrant crystalline U(IV) fraction (corresponding most likely to  $UO_2$ ) was then determined by subtraction of the NCU4 and U(VI) quantity from the total amount of U. Each extraction was repeated at least in triplicates so that the error of the assay is given as a standard deviation amongst the experimental and analytical replicates. Errors were propagated in the case of subtracted values. Aliquots of the digests were filtered through 0.22 µm PTFE filters (ThermoFisher, USA), diluted by an appropriate factor in 0.1 M nitric acid (HNO<sub>3</sub>) and analyzed by ICP-MS for total U extracted from the solids.

In select experiments, XAS analysis of dry sediments was performed at the Stanford Synchrotron Radiation Lightsource (SSRL) for iron (Fe) and sulfur (S) speciation. Fe K-edge spectra were collected at BL 4-1 of SSRL at SLAC. Spectra were collected in transmission mode at 77K in an LN2 cryostat. The energy was selected by a Si(111) double crystal monochromator. The energy was calibrated on the first inflection point of a Fe foil (7,112 eV). The sample was diluted in fructose for optimal signal in transmission mode and pressed in 7 mm diameter pellets protected in Kapton tape and mounted on aluminum holders. S K-edge XAS measurements were conducted in fluorescence mode on ground sediments at BL 4-3 at SSRL with a passivated implanted planar silicon (PIPS) detector at room temperature under a helium atmosphere. The energy for S K-edge was calibrated at the first inflection point of a sodium thiosulfate standard (2,472 eV). XAS data were averaged, normalized and background-subtracted using ATHENA<sup>25</sup> to extract experimental XANES and EXAFS functions. S XANES and Fe EXAFS spectra were interpreted by linear combination fit analyses (LCF) with reference compounds that are presented in Figure SI-1 and Figure SI-2. The quality of the LCF fits was estimated on the R-factor parameter calculated by Athena (Equation SI-1).

#### 3.3.5 Hydrogen peroxide analysis

Hydrogen peroxide ( $H_2O_2$ ) content in the effluent was quantified by the chemiluminescent reaction of acridinium ester (AE, 10-methyl-9-(p-formylphenyl)acridinium carboxylate trifluoromethanesulfonate) and the conjugate base of  $H_2O_2$  ( $HO_2^-$ ,  $pK_a=11.6$ )<sup>26</sup>. As  $H_2O_2$  is the most stable ROS, the net production of  $H_2O_2$  was interpreted as evidence for the presence of ROS in the system. The same AE chemiluminescence (AE-CL) method has been successfully applied both in previous field<sup>16</sup> and laboratory<sup>19,27</sup> studies. Ferrozine was precautionarily used as a ligand to avoid interference by Fe(II)<sup>28</sup>. Chemiluminescence was measured at 470 nM in a 96-well plate reader (Synergy MX, Biotek, USA). 50 µL of ferrozine to a final concentration of 249 µM and 400 µL of AE to a final concentration of 10 µM were added to 1.6 mL of sample. 200 µL aliquots were transferred to the microplates reader. As a final step, 10 µL of buffer solution was added before acquiring the luminescence. The buffer solution was prepared by dissolving 30.1 g of boric acid into 250 mL of 1M NaOH and adjusting to pH 10.2. The AE stock solution was prepared by dissolving 1.6 mg of AE in 10 mL of phosphate buffer at pH 2.6 and stored at -20 °C until use with appropriate dilution in milliQ water.

# 3.4 Results and Discussion

# 3.4.1 U and Fe speciation in the sediments

Regardless of the biogeochemical conditions (iron- or sulfate-reducing conditions), biostimulation of RABS sediments results in the formation of NCU4 as the dominant product. This finding was presented for SRC in Chapter 2 and is also evidenced by the speciation of U via L<sub>III</sub> XANES LCF (Table SI-2 and Figure SI-3) and EXAFS shell by shell fit for IRC6 (Figure SI-4, Figure SI-5 and Table SI-3).

A comparison of the iron speciation in SRC and IRC sediments by LCF of Fe K-edge EXAFS data reveals that while FeS is abundant in SRC sediments, representing 25% of total Fe, it is not detected in RABS or in IRC6 (Figure SI-6 and Table 1). In RABS, the ferric oxide pool accounts for ~53% of the total iron but decreases to ~33% upon biostimulation of RABS in the absence of sulfate (IRC) (Table 1). There is a concomitant increase in the contribution of iron in clays which is due to the fact that the total concentration of iron in IRC is smaller than that in RABS (4.5 vs. 4.83 wt.%) due to the advective transport of aqueous Fe(II) out of the column. A similar loss of aqueous Fe(II) was observed in previous studies that stimulate iron-reducing condition in sediments from Rifle<sup>29</sup>. Hence, because iron is preferentially lost from ferric oxides rather than clays, the relative contribution of Fe in clays increases.

Table 3.1 LCF of the Fe K-edge EXAFS data for RABS as well as in SRC and IRC sediments before oxidation. (-) Reference compound	not required
for the fit. The data for RABS and SRC were already presented in Chapter 2.	

	RABS	SRC	IRC
model compounds	(%)	(%)	(%)
Fe in clays	45	71	59
Fe in ferric oxides	53	4	33
Magnetite	-	0	5
Mackinawite	2	25	-
Siderite	-	-	2
R-factor	0.0153	0.0208	0.0163
Fe content (wt.%)	4.83	5.5	4.5

# 3.4.2 Hydrogen peroxide production

When reduced SRC and IRC sediments are exposed to high (8.56 mg/L) and low (2.14 mg/L) DO, hydrogen peroxide (H2O2) is produced (Figure SI-7). Superoxide (O2–), H2O2 and hydroxyl radical (HO•) are the intermediates generated during the rapid sequential one-electron reduction of O2 to H2O <sup>17,27</sup>. As H<sub>2</sub>O<sub>2</sub> has the longest half-life and is readily analyzed, it is often interpreted as a proxy for the production of all reactive oxygen species (ROS)<sup>16</sup>. Under the conditions investigated, the net production of H<sub>2</sub>O<sub>2</sub> was affected by the dissolved oxygen concentration (DO) and the mineralogy of the sediment (Figure SI-7). A lower DO concentration resulted in an H<sub>2</sub>O<sub>2</sub> concentration 5 to 6-fold lower (Figure SI-7): at 2.14 mg/L DO, the net production of H<sub>2</sub>O<sub>2</sub> in SRC peaked at 5nM, while in IRC it peaked at 10nM. The timing of H<sub>2</sub>O<sub>2</sub> production is comparable with ROS production quantified in batch experiments with phyllosilicates, microbially reduced sediments<sup>14</sup>, and mackinawite<sup>15</sup>. The relevance of ROS as oxidants in nature was

confirmed by a field study that recorded a similar production of  $H_2O_2$  (range 10-54 nM) in the Rifle aquifer in reduced zones characterized by high concentrations of organic carbon and reduced mineral phases including Fe(II)-bearing phases<sup>16</sup>. Previous studies have shown that dark production of ROS in the subsurface is driven by the oxidation of Fe(II) species<sup>14,15,19</sup> through Fenton's and Fenton's like reaction<sup>16,30</sup>. While it is out of the scope of this work to deconvolute the mechanism of  $H_2O_2$  production in SRC and IRC sediments, we propose that the distinct speciation and concentration of Fe in SRC vs. IRC may contribute to the differences in net  $H_2O_2$  production. As expected, amendment of the sediment suspensions with both superoxide dismutase (SOD) and catalase (CAT) resulted in the rapid reduction of ROS to  $H_2O$  and the absence of detectable  $H_2O_2$  (Figure SI-7).

For SRC sediments, the  $H_2O_2$  concentration is lower than that measured in IRC sediments. We do not interpret these data as a higher production of  $H_2O_2$  by IRC sediments because the measurement reflects both sources and sinks of ROS. Hence, these data should be taken to indicate that ROS are produced in both sediments but a comparison between sediments of ROS amount produced is not possible here.

#### 3.4.3 Uranium oxidation in batch experiments

Regardless of the geochemical composition of the sediments investigated, the solution concentration of U in batch experiments increased with time until it reached a constant value after ~8 hours under oxic conditions (Figure 3.1a) and ~10 hours under suboxic conditions (Figure 3.1b). The results clearly show that U is released more rapidly in SRC than in IRC both under oxic and suboxic conditions (Figure 3.1). As the main difference between the SRC and IRC systems is the presence of FeS in SRC, this finding suggests that FeS accelerates U oxidation, which is consistent with the previous report<sup>10</sup>. Furthermore, the concentration of U in sediments treated with SOD and CAT was systematically lower than that in untreated sed-iments (Figure 3.1). This finding indicates that ROS are contributing to U(IV) oxidation as was shown for manganese<sup>23,31</sup>. Because the production of ROS depends on the DO concentration (Figure SI-7), it is

expected that more ROS are produced under oxic conditions and contribute to a greater extent to U(IV) oxidation than in suboxic experiments (Figure 3.1).



Figure 3.1 Uranium aqueous concentration over time during exposure to oxygen in a batch reactor for SRC and IRC sediments with and without SOD and CAT under oxic (a) and suboxic (b) conditions

The results of the quantitative speciation of solid-phase U confirm the observation of U release into solution. Under oxic conditions, NCU4 is consumed rapidly in SRC with only 75 nmoles out of the initial 459 nmoles remaining after three hours of oxidation and only 3 nmoles remaining after 15 hours (Figure 3.2, Table SI-6). In contrast, in the control experiment in which ROS was reduced by SOD and CAT, 193 nmoles of NCU4 remained after three hours of oxidation and 62 nmoles after 15 hours. In IRC, the rate of NCU4 oxidation was slower than in SRC, and the contribution of ROS to U(IV) oxidation appeared to be dramatically lower as there was only a small difference in the amount of NCU4 remaining after 3 hours without and with SOD/CAT (210 nmoles vs. 253 nmoles, respectively) and after 15 hours of oxidation in IRC and IRC with SOD and CAT (100 vs. 129 nmoles respectively) (Table SI-7). Furthermore, the rate of NCU4 oxidation was affected by the DO concentration, as a lower oxidation rate was observed under suboxic conditions (Figure SI-8, Table SI-8) than under oxic conditions for SRC. Differences between the system with and without SOD and CAT are less evident under suboxic conditions, which was attributed to the lower production of ROS due to the lower DO. After three hours of oxidation, NCU4 remaining was 264-278 nmoles in SRC and 241-256 nmoles in IRC. After 15 hours, NCU4 decreased to 158-180 nmoles in SRC and 142-154 nmoles in IRC (Table SI-8 and Table SI-9).



Figure 3.2 Uranium speciation over time during the batch oxidation experiment under oxic conditions for (a) SRC sediments and (b) IRC sediments. Solid lines represent the experiment with no amendment, and dotted lines represent the amendment of SOD and CAT.

The rate of NCU4 oxidation is approximately 30% faster in the presence as compared to the absence of ROS in SRC (128 nmol/hr vs. 88.6 nmol/hr) (Figure 3.1). In contrast, there is little difference in oxidation rate for IRC treated with SOD and CAT vs. untreated, undescoring the role of FeS through ROS in accelerating the oxidation of NCU4. Given the reactivity of ROS, perhaps the difference between SOD and CAT treatment and no treatment would have been expected to be greater. We considered the possibility that FeS was consumed within the first three hours, blunting its impact on U(IV) oxidation.

To do so, we considered the solid phase speciation of S as obtained by K edge XANES. The data demonstrated that mackinawite underwent rapid oxidation in SRC sediments, disappearing by the first sampling point (3 hours). More specifically, the peak corresponding to FeS (at 2,470.3 eV), which dominates the spectrum in the initial SRC sediment, disappeared in the samples starting at 3 hours (Figure 3.3a). The shape and the position of the main peak in the spectrum at 2,472.6 eV after three hours revealed that  $S^0$  was present in the solids. The LCF analyses indicated the contribution of  $S^0$  to the total S in the sediments reached 61% after 3 hours but decreased to 58 and 53% at 6 and 15 hours, respectively (Table 3.2). Even though the difference was smaller than the error of the technique (~10%)<sup>32</sup>, it was considered as relevant because shifts in energy and shape were observed in the spectrum at 3 hours relative to those at 6 and 15 hours<sup>13</sup>. Therefore, S speciation provides evidence for the rapid oxidation of FeS to  $S^0$ , which is subsequently further oxidized to  $SO_4^2$ . In summary, the speciation of U and S in SRC over time are consistent and show rapid oxidation. Hence, the impact of FeS and the ROS produced by its reaction with  $O_2$  is greatest early in the experiment and may have represented a greater increase relative to no ROS (treatment with SOD and CAT) had an earlier time point been available.

The magnitude of the ROS effect in SRC is larger than in IRC, suggesting that FeS significantly contributes to the production of ROS and, indirectly, to the oxidation of NCU4. Indeed, Cheng et al.15 report that, via a two-electron reduction of  $O_2$  to  $H_2O_2$  and a further reduction to •OH, FeS has a greater efficiency of •OH production than other reduced iron minerals (i.e., pyrite, siderite, and Fe0). Thus, we propose that ROS production via the oxidation of FeS is a relevant pathway for NCU4 oxidation. While previous work has reported faster oxidation of NCU4 in the presence of FeS<sup>10</sup>, it invoked a reactive intermediate Fe(III) species that rapidly cycled through oxidation of U(IV) and reduction of  $O_2$  to justify the rapid mobilization of NCU4. Here, we suggest that the oxidation of FeS and Fe(II)-bearing minerals by  $O_2$  drives the dark production of ROS intermediates oxidizing U(IV) as observed for arsenic<sup>14,15</sup> and for other transition metals<sup>18,22,23</sup> but that FeS contributes to the generation of ROS.



Figure 3.3 S K-edge XANES speciation in SRC sediments before and after oxidation under oxic conditions in (a) batch experiments and (b) flowthrough experiments. Solid lines represent the data while dashed lines represent the models.

Table 3.2 results of LCF analysis of S K-edge XANES spectra of SRC sediments before and after oxidatior
in batch experiments under oxic conditions.

	(%) contribution to S speciation							
		Batch ex	periment	ts	Flow-through experiments			
Model compound	Initial (SRC4)	3 hr	ır 6 hr 15 hr		21 hr	68 hr	296 hr	
1. Mackinawite	91	-	-	-	7	2	-	
3. Elemental sulfur	1	61	58	53	51	55	71	
4. S in organics	5	27	32	35	32	36	16	
5. Sulfate	3	13	10	11	10	7	13	
R-factor	0.0184	0.0409	0.0238	0.009	0.0085	0.0083	0.0216	

## 3.4.4 Uranium oxidation in flow-through experiments

In addition to the batch systems, we investigated NCU4 oxidation in flow-through reactors, with a flow rate that is representative of the conditions in the Rifle aquifer (0.8 m/d). U(VI) release was similar for SRC and IRC under both oxic and suboxic conditions (Figure SI-9). The bicarbonate concentration impacted the rate of U release to a greater extent than the sediment type (Figure SI-9). Thus, despite having observed an increase in the rate of NCU4 oxidation associated with the presence of FeS in the batch incubation and the study by Bi et al.<sup>10</sup> having reported the same impact of FeS in well-mixed flow-through reactors, there was no difference between the rate of oxidation of NCU4 in IRC and SRC sediments under oxic flowthrough conditions. The difference between batch and flow-through experiments is attributable to the distinct total amount of oxygen available in the two systems. We calculate that within the first 15 hours of the oxic flow-through experiment, there is a lower total amount of O<sub>2</sub> available than in the batch suboxic experiment (0.25 vs 3.60 mg, respectively). This is because while the influx of  $O_2$  in the flow-through reactor is limited by the flow rate, in the batch experiment, the equilibration of the aqueous phase with the headspace allows the partitioning of  $O_2$  from the gas phase. Therefore, in flow-through experiments, the role of FeS is negligible due to the low amount of O<sub>2</sub> available for the production of ROS and there are no notable differences between the concentrations of U from the outflow of the SRC and IRC flow-through systems.

Results from quantitative speciation of solid-associated U are consistent with the aqueous U results as the rate of NCU4 oxidation in SRC and IRC are found to be similar (Figure 3.4, Table SI-10, Table SI-11). Under oxic conditions, NCU4 was rapidly oxidized with 0.45 and 0.53 µmoles NCU4 remaining after 21 hours from the initial 1.4 or 1.3 µmoles for SRC and IRC, respectively and little NCU4 remaining after 296 hours (70 and 80 nmoles, respectively).



Figure 3.4 Uranium speciation over time during the flow-though oxidation experiment at 10 mM HCO<sub>3</sub> under (a) oxic and (b) suboxic conditions in SRC sediments (solid lines) and IRC sediments (dashed lines).

Rapid oxidation of FeS in SRC sediments was evidenced by analysis of Fe K-edge EXAFS as the contribution of the distinctive FeS feature in the Fe EXAFS, observed in the original sediments between k = 7.5 and 8.5, decreased over time (Figure 3.5). Based on LCF results, the FeS contribution to Fe speciation in SRC decreased from 25% to 8% after 21 hours and further to 2% after 296 hours (Table 3). At the same time, the contribution of ferric oxides increased from 4% to 50% after 21 hours and to 60% after 296 hours. Fe transformation in IRC sediments followed a similar pattern as in SRC: over 296 hours, the contribution of Fe in ferric oxides increased from 33% to ~53%. LCF also indicated an increase in the contribution of magnetite (Fe<sub>3</sub>O<sub>4</sub>) over time in both SRC and IRC to a final concentration of 14% (from 0%) in SRC and 22% (from 5%) in IRC. After undergoing reduction (RABS to SRC and IRC) followed by oxidation (296 hours), the speciation of iron in the sediments resembles the original mineralogy with the exception of a greater contribution of magnetite than in RABS and a smaller contribution of iron in clays, suggesting the release of iron from clays (Table 3.3).

# Table 3.3 Fe K-edge EXAFS speciation in SRC and IRC sediments before and after oxidation under oxic flow-through experiments. The data for RABS and the initial composition of SRC were already presented in Chapter 2.

		SRC			IRC				
	RABS	Initial SRC	Flow-through experi-			Initial IRC	Flow-through experi-		
			ment				ment		
model com-		0 hr	21 hr	68 hr	296 hr	0 hr	21 hr	68 hr	296 hr
pounds									
Fe in clays	45	71	43	38	23	59	40	39	22
Fe in ferric	53	4	50	52	60	33	51	52	53
oxide									
Magnetite	-	0	0	6	14	5	2	4	22
Mackinawite	2	25	8	5	2	-	-	-	-
Siderite	-	-	-	-	-	2	7	5	4
R-factor	0.015	0.0208	0.0110	0.0052	0.0068	0.0163	0.085	0.0069	0.0046



Figure 3.5 Fe K Edge EXAFS speciation before and after oxidation in CSTR experiment for SRC (a) and IRC (b) sediments The contribution of FeS to the total pool of S in SRC decreased from 91%, in the reduced sediment before oxidation, to 7% after 21 hours and 2% at 68 hours (Table 3.2). FeS was no longer detected at 296 hours. Concomitantly, the content of S<sup>0</sup> increased from 1% in the initial SRC to 51% at 21 hours, 55% at 68 hours, and 71% at 296 hours. Furthermore, water chemistry analyses revealed that  $SO_4^{2-}$  dissolution occurred at two different rates: initial slow dissolution in the first 150 hours and rapid dissolution at the end of the experiment (Figure SI-10). In the first 150 hours, only 1.9-2% of the total sediment S was detected as dissolved S-SO<sub>4</sub><sup>2-</sup>. After 150 hours, there was a change in the rate of increase of dissolved S-SO<sub>4</sub><sup>2-</sup> as 15-17% of total S was released as S-SO<sub>4</sub><sup>2-</sup> from the sediment between 150 hours and 300 hours (Figure SI-10). Thus, the oxidation of FeS in SRC occurred in two consecutive steps: first, the rapid oxidation of FeS to S<sup>0</sup> which accumulated in the solid phase in the first ~150 hours of the experiment with limited oxidation to sulfate and second, the slow oxidation of S<sup>0</sup> to soluble SO<sub>4</sub><sup>2-</sup> which was lost to the effluent (Figure SI-11).

The overall picture that emerges from the oxidation of IRC and SRC in flow-through systems is the concomitant oxidation of FeS or Fe(II)-bearing phases and NCU4, as was observed in the batch experiment,

despite the absence of a contribution of ROS. We conclude that, under these conditions, the oxidation is directly mediated by dissolved  $O_2$ . Interestingly, in the absence of ROS, FeS has no impact on the rate of oxidation of NCU4 relative to other Fe(II)-bearing phases, not even a protective effect as is observed for  $UO_2^{33}$ .

# 3.5 Environmental implications

FeS has largely been investigated for its capacity to reduce contaminants in the environment. During *in situ* bioreduction, when sulfate-reducing conditions are established, FeS rapidly immobilizes U(VI), and it is commonly reported in association with U(IV). In general, the effect of FeS oxidation by incoming O<sub>2</sub> on the speciation of U has been largely overlooked. However, in the subsurface, it is expected that fluctuation of redox conditions create oxic/anoxic interfaces where ROS are likely to be generated<sup>16</sup> at different rates depending on the speciation of reduced iron<sup>15</sup> and the organic carbon content<sup>27</sup>. Here, we demonstrate that FeS indirectly accelerates the oxidation and mobilization of non-crystalline U(IV) through the formation of ROS. However, the formation of ROS strongly depends on the concentration of dissolved O<sub>2</sub>. Hence, if low DO concentrations prevail, little ROS will be produced and FeS will not impact NCU4 stability. In contrast, if DO concentrations rise, we predict that FeS will multiply the impact of high DO by catalysing the formation of ROS, with the attendant rapid oxidation of NCU4, to a greater extent than other Fe(II)-bearing phases. Hence, in U-bearing reduced sediments undergoing oxic-anoxic oscillations, the impact of the local DO concentration will be multiplied by the presence of FeS.

# 3.6 Acknowledgments

This study is funded by the Swiss National Science Foundation under Grant # 200020-144335. The S Kedge and Fe K-edge XAS experiments were performed on the 4-3 and 4-1 beamline at the Stanford Synchrotron Radiation Lightsource (SSRL). We are grateful for technical assistance received during the XAS analyses from Ryan Davis, Erik Nelson, and Matthew Latimer. Use of the SSRL, SLAC National Accelerator Laboratory is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract DE-AC02-76SF00515. We thank Vincent Noel for sharing Phlogopite and Mackinawite model compounds for the XAS analysis of Fe and S data.

# 3.7 References

(1) Tong, M.; Yuan, S.; Ma, S.; Jin, M.; Liu, D.; Cheng, D.; Liu, X.; Gan, Y.; Wang, Y. Production of Abundant Hydroxyl Radicals from Oxygenation of Subsurface Sediments. *Environ. Sci. Technol.* **2016**, *50* (1), 214–221. https://doi.org/10.1021/acs.est.5b04323.

(2) Cheng, D.; Yuan, S.; Liao, P.; Zhang, P. Oxidizing Impact Induced by Mackinawite (FeS) Nanoparticles at Oxic Conditions Due to Production of Hydroxyl Radicals. *Environ. Sci. Technol.* **2016**, *50* (21), 11646–11653. https://doi.org/10.1021/acs.est.6b02833.

(3) Lovley, D. R.; Phillips, E. J. P.; Gorby, Y. A.; Landa, E. R. Microbial Reduction of Uranium. *Nature* **1991**, *350* (6317), 413. https://doi.org/10.1038/350413a0.

(4) Veeramani, H.; Alessi, D. S.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Sharp, J. O.; Dippon, U.; Kappler, A.; Bargar, J. R.; Bernier-Latmani, R. Products of Abiotic U(VI) Reduction by Biogenic Magnetite and Vivianite. *Geochimica et Cosmochimica Acta* **2011**, *75* (9), 2512–2528. https://doi.org/10.1016/j.gca.2011.02.024.

(5) Williams, K. H.; Bargar, J. R.; Lloyd, J. R.; Lovley, D. R. Bioremediation of Uranium-Contaminated Groundwater: A Systems Approach to Subsurface Biogeochemistry. *Current Opinion in Biotechnology* **2013**, *24* (3), 489–497. https://doi.org/10.1016/j.copbio.2012.10.008.

(6) 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. Non-Uraninite Products of Microbial U(VI) Reduction. *Environ. Sci. Technol.* 2010, 44 (24), 9456–9462. https://doi.org/10.1021/es101675a.

(7) Bargar, J. R.; Williams, K. H.; Campbell, K. M.; Long, P. E.; Stubbs, J. E.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Alessi, D. S.; Stylo, M.; Webb, S. M.; et al. Uranium Redox Transition Pathways in Acetate-Amended Sediments. *PNAS* **2013**, *110* (12), 4506–4511. https://doi.org/10.1073/pnas.1219198110.

(8) Alessi, D. S.; Lezama-Pacheco, J. S.; Janot, N.; Suvorova, E. I.; Cerrato, J. M.; Giammar, D. E.; Davis, J. A.; Fox, P. M.; Williams, K. H.; Long, P. E.; et al. Speciation and Reactivity of Uranium Products Formed during in Situ Bioremediation in a Shallow Alluvial Aquifer. *Environ. Sci. Technol.* **2014**, *48* (21), 12842–12850. https://doi.org/10.1021/es502701u.

Bhattacharyya, A.; Campbell, K. M.; Kelly, S. D.; Roebbert, Y.; Weyer, S.; Bernier-Latmani, R.; Borch,
 T. Biogenic Non-Crystalline U<sup>(IV)</sup> Revealed as Major Component in Uranium Ore Deposits. *Nature Communications* 2017, *8*, 15538. https://doi.org/10.1038/ncomms15538.

Noël, V.; Boye, K.; Lezama Pacheco, J. S.; Bone, S. E.; Janot, N.; Cardarelli, E.; Williams, K. H.; Bargar, J. R. Redox Controls over the Stability of U(IV) in Floodplains of the Upper Colorado River Basin. *Environ. Sci. Technol.* 2017, *51* (19), 10954–10964. https://doi.org/10.1021/acs.est.7b02203.

(11) Cerrato, J. M.; Ashner, M. N.; Alessi, D. S.; Lezama-Pacheco, J. S.; Bernier-Latmani, R.; Bargar, J. R.;
 Giammar, D. E. Relative Reactivity of Biogenic and Chemogenic Uraninite and Biogenic Noncrystalline
 U(IV). *Environ. Sci. Technol.* 2013, 47 (17), 9756–9763. https://doi.org/10.1021/es401663t.

(12) Bi, Y.; Stylo, M.; Bernier-Latmani, R.; Hayes, K. F. Rapid Mobilization of Noncrystalline U(IV) Coupled with FeS Oxidation. *Environ. Sci. Technol.* **2016**, *50* (3), 1403–1411. https://doi.org/10.1021/acs.est.5b04281.

(13) Bi, Y.; Hayes, K. F. Surface Passivation Limited UO2 Oxidative Dissolution in the Presence of FeS. *Environ. Sci. Technol.* **2014**, *48* (22), 13402–13411. https://doi.org/10.1021/es5041392.

(14) Alessi, D. S.; Uster, B.; Veeramani, H.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Bargar, J. R.; Bernier-Latmani, R. Quantitative Separation of Monomeric U(IV) from UO2 in Products of U(VI) Reduction. *Environ. Sci. Technol.* 2012, *46* (11), 6150–6157. https://doi.org/10.1021/es204123z.

(15) Noël, V.; Boye, K.; Kukkadapu, R. K.; Bone, S.; Lezama Pacheco, J. S.; Cardarelli, E.; Janot, N.; Fendorf, S.; Williams, K. H.; Bargar, J. R. Understanding Controls on Redox Processes in Floodplain Sediments of the Upper Colorado River Basin. *Science of The Total Environment* **2017**, *603–604*, 663–675. https://doi.org/10.1016/j.scitotenv.2017.01.109.

Yuan, X.; Nico, P. S.; Huang, X.; Liu, T.; Ulrich, C.; Williams, K. H.; Davis, J. A. Production of Hydrogen Peroxide in Groundwater at Rifle, Colorado. *Environ. Sci. Technol.* 2017, *51* (14), 7881–7891. https://doi.org/10.1021/acs.est.6b04803.

(17) Zhang, T.; Hansel, C. M.; Voelker, B. M.; Lamborg, C. H. Extensive Dark Biological Production of Reactive Oxygen Species in Brackish and Freshwater Ponds. *Environ. Sci. Technol.* **2016**, *50* (6), 2983–2993. https://doi.org/10.1021/acs.est.5b03906.

71

(18) Page, S. E.; Kling, G. W.; Sander, M.; Harrold, K. H.; Logan, J. R.; McNeill, K.; Cory, R. M. Dark Formation of Hydroxyl Radical in Arctic Soil and Surface Waters. *Environ. Sci. Technol.* **2013**, *47* (22), 12860– 12867. https://doi.org/10.1021/es4033265.

(19) Murphy, S. A.; Solomon, B. M.; Meng, S.; Copeland, J. M.; Shaw, T. J.; Ferry, J. L. Geochemical Production of Reactive Oxygen Species From Biogeochemically Reduced Fe. *Environ. Sci. Technol.* **2014**, *48* (7), 3815–3821. https://doi.org/10.1021/es4051764.

(20) Zhang, P.; Yuan, S.; Liao, P. Mechanisms of Hydroxyl Radical Production from Abiotic Oxidation of Pyrite under Acidic Conditions. *Geochimica et Cosmochimica Acta* **2016**, *172*, 444–457. https://doi.org/10.1016/j.gca.2015.10.015.

(21) Voelker, B. M.; Barbara Sulzberger. Effects of Fulvic Acid on Fe(II) Oxidation by Hydrogen Peroxide
Environmental Science & Technology (ACS Publications) (accessed Jun 7, 2018).

(22) Pham, A. N.; Xing, G.; Miller, C. J.; Waite, T. D. Fenton-like Copper Redox Chemistry Revisited: Hydrogen Peroxide and Superoxide Mediation of Copper-Catalyzed Oxidant Production. *Journal of Catalysis* **2013**, *301*, 54–64. https://doi.org/10.1016/j.jcat.2013.01.025.

(23) Learman, D. R.; Voelker, B. M.; Vazquez-Rodriguez, A. I.; Hansel, C. M. Formation of Manganese Oxides by Bacterially Generated Superoxide. *Nature Geoscience* **2011**, *4* (2), 95–98. https://doi.org/10.1038/ngeo1055.

(24) Komlos, J.; Peacock, A.; Kukkadapu, R. K.; Jaffé, P. R. Long-Term Dynamics of Uranium Reduction/Reoxidation under Low Sulfate Conditions. *Geochimica et Cosmochimica Acta* **2008**, *72* (15), 3603– 3615. https://doi.org/10.1016/j.gca.2008.05.040.

(25) Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: Data Analysis for X-Ray Absorption Spectroscopy Using IFEFFIT. *J Synchrotron Radiat* **2005**, *12* (Pt 4), 537–541. https://doi.org/10.1107/S0909049505012719.

(26) Cooper, W. J.; Moegling, J. K.; Kieber, R. J.; Kiddle, J. J. A Chemiluminescence Method for the Analysis of H2O2 in Natural Waters. *Marine Chemistry* **2000**, *70* (1), 191–200. https://doi.org/10.1016/S0304-4203(00)00025-6. (27) Yuan, X.; Davis, J. A.; Nico, P. S. Iron-Mediated Oxidation of Methoxyhydroquinone under Dark Conditions: Kinetic and Mechanistic Insights. *Environ. Sci. Technol.* **2016**, *50* (4), 1731–1740. https://doi.org/10.1021/acs.est.5b03939.

(28) Vermilyea, A. W.; Voelker, B. M. Photo-Fenton Reaction at Near Neutral PH. *Environ. Sci. Technol.* **2009**, 43 (18), 6927–6933. https://doi.org/10.1021/es900721x.

Moon, H. S.; McGuinness, L.; Kukkadapu, R. K.; Peacock, A. D.; Komlos, J.; Kerkhof, L. J.; Long, P.
 E.; Jaffé, P. R. Microbial Reduction of Uranium under Iron- and Sulfate-Reducing Conditions: Effect of Amended Goethite on Microbial Community Composition and Dynamics. *Water Research* 2010, 44 (14), 4015–4028. https://doi.org/10.1016/j.watres.2010.05.003.

(30) King, D. W.; Lounsbury, H. A.; Millero, F. J. Rates and Mechanism of Fe(II) Oxidation at Nanomolar
Total Iron Concentrations. *Environmental Science & Technology* 1995, *29* (3), 818–824.
https://doi.org/10.1021/es00003a033.

(31) Nico, P. S.; Anastasio, C.; Zasoski, R. J. Rapid Photo-Oxidation of Mn(II) Mediated by Humic Substances. *Geochimica et Cosmochimica Acta* **2002**, *66* (23), 4047–4056. https://doi.org/10.1016/S0016-7037(02)01001-3.

(32) Prietzel, J.; Botzaki, A.; Tyufekchieva, N.; Brettholle, M.; Thieme, J.; Klysubun, W. Sulfur Speciation in Soil by S K-Edge XANES Spectroscopy: Comparison of Spectral Deconvolution and Linear Combination Fitting. *Environ. Sci. Technol.* **2011**, *45* (7), 2878–2886. https://doi.org/10.1021/es102180a.

(33) Bi, Y.; Hyun, S. P.; Kukkadapu, R. K.; Hayes, K. F. Oxidative Dissolution of UO2 in a Simulated Groundwater Containing Synthetic Nanocrystalline Mackinawite. *Geochimica et Cosmochimica Acta* **2013**, *102*, 175–190. https://doi.org/10.1016/j.gca.2012.10.032.

# Chapter 4 Uranium isotope fractionation during reduction of U(VI) by magnetite

This chapter presents the results of preliminary experiments that probe the isotopic fractionation of U during reduction in batch experiments using synthetic magnetite nanoparticles as reductants. We present the results from the isotopic fractionation measurements in the aqueous phase, and the speciation of U immobilized on the solids at the end of the reaction by L<sub>III</sub>-edge X-ray absorption spectroscopy.

*Luca Loreggian*; experimental work and associated wet chemistry (ICP-MS) and mineralogical (XAS) analysis. *Yvonne Roebert;* MC ICP-MS analysis. *Barbora Bartova*; characterization of magnetite (STEM). *Rizlan Bernier-Latmani;* conceptual guidance.

# 4.1 Abstract

The ratio of the two major isotopes ( $^{238}$ U/ $^{235}$ U) varies in natural environments depending on biogeochemical conditions. Recently, the use of  $^{238}$ U/ $^{235}$ U has been investigated in a few field-studies with the purpose of exploring its potential use as a tracer for U immobilization at contaminated sites using the large isotopic shifts that occur during U(VI) reduction to U(IV). Although  $^{238}$ U/ $^{235}$ U has been proposed as a marker for U reduction, a deeper understanding of the mechanism of fractionation is required prior to its application in the field. In this regard, this work aims to investigate the U isotopes fractionation during reduction of U(VI) by magnetite that is a ubiquitous Fe(II)-mineral in reduced zones.

We show that abiotic reduction by magnetite exhibits the opposite fractionation than biotic reduction (i.e., preferential reduction of <sup>235</sup>U) and that the isotopic shift regularly appears in the second phase of reaction when the reduction rate slows down compared to the initial rapid reaction in the first phase. In addition, we observe that U loading on  $Fe_3O_4$  impacts on the extent of fractionation. In fact, the higher fractionation effect occurs at high U loading when the reduction rate is slower than at low U loading. To conclude, we demonstrate that, although adsorption has a similar isotopic signature than reduction, the extent of fractionation is smaller than reduction. Hence, adsorption prior to reduction contributes to a small extent and reduction itself is the process responsible for the extreme shifts that are reported in this study.

Furthermore, the analysis of the solid phase U revealed that, under specific conditions, a significant amount of U(V) accumulates and persists on the surface of magnetite. This finding represents the first evidence of stable U(V) at neutral pH on the surface of magnetite, but the role of U(V) in the fractionation of U(VI) to U(IV) remains to be investigated.

KEYWORDS: uranium, isotopes, fractionation, magnetite, abiotic reduction

# 4.2 Introduction

The ratio of the two major uranium (U) isotopes, <sup>238</sup>U and <sup>235</sup>U, varies in natural environments depending on the biogeochemical conditions<sup>1</sup>. Uranium isotopes have shown a potential for monitoring the remediation of U ore mines<sup>5</sup>. In fact, a few studies have recently investigated the use of U isotopes fractionation to trace the mobility of U in the groundwater after mining has ceased using the comparatively large isotopic shifts that occur during U(VI) reduction to U(IV). For example, Basu et *al.*<sup>6</sup> and Brown et *al.*<sup>7</sup> observed a large isotopic fractionation from nearby ISR mining sites respectively of more than 3‰ in groundwater at Rosita and Kingsville Dome Texas, USA and 0.78-1.03‰ at Smith Ranch Texas, USA. A similar isotopic signature was observed at the Old Rifle site during an in-situ remediation trial using an organic carbon amendment to stimulate microbial processes<sup>8</sup>. Although these studies demonstrate that it is possible to use U isotopes as a tracer for U reduction in the field, they also revealed a significant variation in the isotopic shift which is likely due to different processes with different isotopic signature that 'compete' for the removal of U such as adsorption and reduction or abiotic and biotic reduction.

Hence, prior to applying U isotopes as a reliable tracer in the field to deconvolute what is the process that immobilizes U, the accurate understanding of the mechanism of fractionation in different reductive pathways is required. In this regard, a significant gap of knowledge is represented by the difference between biotic and abiotic U isotopes fractionation. A key question to address is what causes of the observed differences between biotic and abiotic U isotopes fractionation are. In this regard, one significant gap of knowledge is represented by the mechanism of fractionation are. In this regard, one significant gap of knowledge is represented by the mechanism of fractionation during abiotic reduction by Fe(II)-bearing minerals such as magnetite. A recent study observed that abiotic reduction by iron-oxides and aqueous Fe(II) exhibits the opposite fractionation (i.e., preferential reduction of <sup>235</sup>U) than in biotic reduction and predicted by theoretical calculation on the nuclear field shift effect. Thus, Stylo et *al.*<sup>3</sup> suggest that this isotopic effect is driven by a kinetic isotope effect. Furthermore, it is observed that U(VI) reduction by

magnetite occurs in two phases: an initial phase during which no fractionation occurs, and a second phase characterized by a slower reaction rate and preferential reduction of the light isotope. Thus, it was hypothesized that the difference in isotopes fractionation depends on the availability of Fe(II) at the mineral surface<sup>3</sup>. According to this hypothesis; since in the initial phase there is abundant Fe(II) available, two consecutive one-electron transfers transform U(VI) to U(IV). In the second phase, Fe(II) becomes limiting, and a one-electron transfer to U(V) is more likely with U(V) that either persists or disproportionates to U(IV) and U(VI). If this hypothesis holds, it will follow that the fraction of U(V) increases late along the reaction progress and that increase may correspond to the isotopic fractionation observed. Finally, this hypothesis implies that U in the products is effectively sequestered so that no isotopic exchange occurs between reduced U and the pool of unreacted U(VI).

In this study, we aim to shed light on the mechanism of U isotopes fractionation during abiotic reduction mediated by magnetite (Fe<sub>3</sub>O<sub>4</sub>). The goals of the work are: (i) to confirm that Fe<sub>3</sub>O<sub>4</sub> preferentially reduces <sup>235</sup>U as shown in a previous study<sup>3</sup>, (ii) to investigate the effect of U loading and reduction rates, (iii) to investigate the isotopic fractionation during U(VI) adsorption on maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) as non-reactive proxy for magnetite, and (iv) to investigate the effect of U(IV) speciation using magnetite that was presorbed with phosphate to favour the formation of non-crystalline U(IV) species<sup>9</sup>. For these purposes, we monitor U(VI) reduction by Fe<sub>3</sub>O<sub>4</sub> and adsorption on  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> in a series of batch incubation experiments under various conditions. We capture the reduction progress over time by quantifying the amount of unreacted U(VI) with inductively coupled mass spectrometry (ICP-MS) after bicarbonate extraction as described by Alessi et *al*.<sup>10</sup>. The isotopic fractionation is systematically probed on the U(VI) pool (after bicarbonate extraction as for total U) with multi-collector ICP-MS (MC ICP-MS). We use X-ray absorption spectroscopy (XAS) to characterize the oxidation state and the coordination environment in the end-products of reduction as well as at intermediate time points in a selected experiment.

# 4.3 Materials and Methods

#### 4.3.1 Synthesis of magnetite and maghemite

The synthesis of Fe<sub>3</sub>O<sub>4</sub> was performed in an anaerobic chamber (Coy Laboratory Products Inc, USA) with an atmosphere of 3%: 97% H<sub>2</sub>: N<sub>2</sub>. All reagents used were >99.9% purity level. All solutions were prepared with milli-Q water and was deoxygenated by purging with N<sub>2</sub>. Fe<sub>3</sub>O<sub>4</sub> nanoparticles were synthesized via a co-precipitation method with a protocol modified from Wang et *al.* <sup>11</sup>. Briefly, 10 mL of 1 M FeCl<sub>2</sub> and 20 mL of 1 M FeCl<sub>3</sub> were mixed in a glass beaker on a magnetic stirrer and homogenized by continuously stirring the solution with a stir bar. The pH of the solution was slowly and steadily increased by titration of 1 M NaOH until pH 11 was reached. The resulting black suspension was transferred in an anaerobic bottle, sealed and incubated overnight at 30 °C and continuously shaken at 140 rpm. The solids were harvested by centrifugation at 10,000g for 20 min and washed twice with anoxic milli-Q water. The wet pellet was dried under vacuum in a desiccant under anoxic conditions. The resulting powder was ground and preserved in the anaerobic chamber for further use. Before use, the ground powder of Fe<sub>3</sub>O<sub>4</sub> was resuspended in milli-Q water and sonicated 3 times for 10 minutes. The suspension was cooled in ice between each sonication to minimize overheating.

Magnetite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) was synthesized by calcination in the air of Fe<sub>3</sub>O<sub>4</sub> nanoparticles at 300 °C for 2 hours in a muffle furnace as in <sup>12</sup>.

#### 4.3.2 Characterization of magnetite

The sample was prepared by suspending the powder in ethanol by ultrasonication and drying a drop of the suspension on a carbon-coated copper grid. In this study, an FEI Tecnai Osiris microscope (200 kV X-FEG field emission gun) was used to acquire High Angle Annular Dark field images in STEM mode in order to obtain images of magnetite particles with high contrast. Hundreds of particles were subsequently analyzed by FIJI<sup>13</sup> to obtain size distribution. Selected area diffraction pattern (SAED) was acquired for all studied samples in order to confirm the crystal structure of magnetite 9013529)<sup>14</sup>.

#### 4.3.3 Uranium adsorption and reduction experiments

U reduction by Fe<sub>3</sub>O<sub>4</sub> is investigated in batch incubation experiments under anoxic atmosphere (%: 97% H<sub>2</sub>: N<sub>2</sub>) in a glove box (Coy Laboratory Products Inc, USA) using sterile acid washed serum bottles with a butyl rubber septum and anoxic pH-buffered (pH =7) solution containing: 20 mM piperazine -N,N'-bis(2-ethanesulfonic)acid (PIPES), 1 mM NaHCO<sub>3</sub> and various concentration of uranyl with chloride as the counteranion. Chemogenic magnetite was dosed as a concentrated suspension (100 mg Fe<sub>3</sub>O<sub>4</sub>/mL) to initiate the reaction. The concentration of Fe is 5 mM Fe as Fe<sub>3</sub>O<sub>4</sub> in all experiments (i.e., 11.57 mg of Fe<sub>3</sub>O<sub>4</sub> in 30 mL volume). To investigate the effect of U loading, U(VI) reduction was tested at two U:Fe molar ratios; 0.04 and 0.016, corresponding to an initial concentration of 200 and 80  $\mu$ M U respectively. The progressive loading of U on the mineral affects the rate of reaction in different experiments that are otherwise identical. This allows us to explore how the isotopic effect change at fast and slow reaction rates. The progress of the reaction and the isotopic fractionation was monitored by measuring the remaining pool of U(VI) after chemical extraction with bicarbonate. Briefly, 500  $\mu$ L aliquot was withdrawn from the batch and incubated in an equal volume of 200  $\mu$ M anoxic NaHCO<sub>3</sub> for 12 hours. The supernatant was then filtered through 0,22  $\mu$ m PTFE filters (ThermoFisher, USA) before analysis for total U concentration with ICP MS and isotopic fractionation coefficient with MC ICP MS.

To investigate the role of U(IV) speciation in the  $\delta^{238}$ U over time, we conducted a reduction experiment using Fe<sub>3</sub>O<sub>4</sub> that was presorbed with phosphate (PO<sub>4</sub><sup>2-</sup>). In fact, it is known that pre-sorption of PO<sub>4</sub><sup>2-</sup> onto the surface of magnetite promotes the formation of less crystalline U(IV) products at the end of reduction<sup>9</sup>. The U:Fe molar ratio was 0.016 corresponding to 5mM of Fe as Fe<sub>3</sub>O<sub>4</sub> and 80 µM of U. To investigate the contribution of adsorption to fractionation, we conducted two batch experiments using  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> as a proxy for non-reactive magnetite. The total content of Fe was the same as in Fe<sub>3</sub>O<sub>4</sub> experiments (i.e., 5 mM Fe as  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>). U(VI) adsorption was investigated at the following U:Fe ratios: 0.28 and 0.16, corresponding to 140 and 80  $\mu$ M of U respectively. In this case, total U and  $\delta^{238}$ U were measured on the filtered supernatant without bicarbonate extraction.

Lastly, a series of batch experiments were conducted with the purpose of monitor changes of U speciation in the solids over time and, simultaneously, monitor the isotope fractionation of remaining U(VI) in the supernatant. The U:Fe molar ratio was set at 0.016 corresponding to 5mM of Fe as  $Fe_3O_4$ , and 80  $\mu$ M of U. U reduction was conducted in steriflips from MerckMillipore (DE). At every time point, the entire content of one batch experiment was filtered through Polyether-sulfone membrane filters of the steriflip. The solid phase was preserved at -80 °C for U speciation by XAS at L<sub>III</sub> edge and the filtrate was preserved for ICP MS and MC ICP MS measurements.

## 4.3.4 MC ICP-MS uranium analysis

All samples were evaporated and treated by a mixture of 400 μL 32% H<sub>2</sub>O<sub>2</sub> and 14 M HNO<sub>3</sub> (1:1). U was purified by ion-exchange chromatography with Eichrom UTEVA resin according to Weyer et *al*. <sup>1</sup>. A standard solution of IRMM 3636-A <sup>236</sup>U/<sup>233</sup>U double spike (10, 39) was added, to correct for isotope fractionation during purification and for instrumental mass discrimination during analyses with multi-collector inductively coupled plasma source mass spectrometer (MC-ICP-MS). Similar spike/sample ratios, corresponding to <sup>236</sup>U/<sup>235</sup>U in the spiked sample mix (within ±10%) were used for all samples and spiked in-run standard. An aliquot of about 400 ng U was used for MS analysis, and U isotopic composition was measured with a Thermo Neptune MC ICP-MS at the Institute for Mineralogy at Leibniz Universität Hannover. For sample introduction, a desolvation unit of a Cetac Aridus-II combined with a 100 μl PFA nebulizer was used at Hannover. The U isotopes (<sup>233</sup>U, <sup>235</sup>U, <sup>236</sup>U, <sup>236</sup>U) were measured on Faraday

cups, connected to 10<sup>11</sup>ohm amplifiers each with a dynamic range of 0 – 50 V. All samples and standards were measured in 70 cycles (each with an integration time of 4 seconds). A standard Ni sampler cone and a Ni X skimmer cone were used. Samples were measured using a sample-standard bracketing method (i.e., every two samples were bracketed by the double-spiked in-run isotopic standard). The in-run standard was IRMM-184. IRMM-184 has an <sup>238</sup>U/<sup>235</sup>U isotope composition of 137.68<sup>1,15</sup>. The accuracy and precision were determined by replicate analyses of various U standards REIMP 18A (Regular European Inter-Laboratory Measurement Evaluation Programme) and CRM-112A. The reproducibility of these standards was about 0.05‰ and the results matched within those previously reported in the literature<sup>1,15</sup>. The abundance sensitivity of the mass spectrometer was checked before each sample analysis session. The results for all sample analyses are reported in the delta notation relative to the IRMM-184:

$$\delta^{238}U = \left[\frac{(^{238}U/^{235}U)_{sample}}{(^{238}U/^{235}U)_{IRMM-184}} - 1\right] * 1000 \ [\%_0]$$

Each sample was analyzed three times, and the precision is reported as two standard deviations (2 S.D.) of the replicate analyses for each sample (typically  $\leq 0.1\%$ ).

#### 4.3.5 X-ray absorption spectroscopy measurements

XAS was used to investigate the oxidation state and coordination environment of U at the end of the reduction or in intermediate solid samples in one selected experiment. U L<sub>III</sub>-edge quick-EXAFS data were collected in fluorescence mode at beamline (BL) B18 at Diamond Light Source (DLS), UK. Fluorescence signal was collected with a 9 elements Germanium (Ge) detector (Canberra industries, USA). The samples were prepared by filtration of the thick suspension of U adsorbed on Fe<sub>3</sub>O<sub>4</sub> nanoparticles through Polyethersulfone membrane filters with 0.22  $\mu$ m porous size in steriflip devices (Merck Millipore, Germany) or via filtration unit. The filter holding on the solids was taped on Kapton tape, enclosed with the Kapton tape and allocated in Nalgene cryovials for measurements at B18. Sample preparation was conducted in

anoxic conditions (3%: 97% H2: N2). Samples were shipped to DLS in a hermetically sealed stainless-steel shipping canister (Schuett-Biotec GmbH, Gottingen, Germany). XAS analysis was conducted at 77 K in liquid nitrogen (LN2) cryostats to reduce beam damage, preserve air-sensitive samples from oxidation and improve data quality. The calibration of the energy was performed on the first inflection point of Yttrium (Y) foil reference (1,7038 eV), that was placed between I<sub>1</sub> and I<sub>2</sub> ion chambers. The same Y foil was used as a calibrant by collecting its transmission signal in I<sub>2</sub> simultaneously to the samples analyzed for the entire duration of the experiment.

#### 4.3.6 XAS Data Analysis

Multiple scans per sample were required to minimize noise to signal ratio. U XAS data were averaged, rebinned, normalized and background-subtracted using ATHENA<sup>16</sup> to extract experimental XANES and EX-AFS functions. U L<sub>III</sub> edge XANES spectra were analyzed by linear combination fit analysis (LCF) with reference compounds that are presented in the (Figure SI-1). The quality of the LCF fits was estimated on the R-factor parameter calculated by Athena<sup>16</sup>. The quality of the fits was estimated by the reduced R-factor (Equation SI 1) and  $X_r^2$  (Equation SI 2).

# 4.4 Results and discussion

#### 4.4.1 Uranium reduction and isotope fractionation

U reduction, when mediated by magnetite under the investigated conditions, occurs in two phases: an initial short phase where the majority of U(VI) is reduced rapidly and a second phase that is characterized by slower reduction rate (Figure 4.1a and Figure 4.1c), which is consistent with a previous study<sup>3</sup>. MC ICP-MS measurements indicate that no isotope fractionation occurs in the first phase of the reaction, but <sup>235</sup>U is preferentially reduced in the second phase. Preferential reduction of <sup>235</sup>U results in positive  $\delta^{238}$ U in the unreacted U(VI) fraction (Figure 4.1b and Figure 4.1d). Independently of the initial concentration of U,

 $δ^{238}$ U begins to increase when ~80% of reduction is completed (i.e., 21% at 200 μM U and 17% at 80 μM U). The largest fractionation was observed at the highest U loading (i.e., 0.04 U:Fe) where  $δ^{238}$ U increased to 5.46±0.03‰ and 5.58±0.03‰ when 8.19 and 8.53 μM of U(VI) is unreacted, corresponding to 3.9% and 4.2% of the initial U(VI) content (Figure 4.1b). Despite the fact that the experiment at low U loading (i.e., 0.016 U:Fe) are less reproducible than the case at U loading, we report a similar isotopic fractionation behavior: no fractionation in the first phase of reaction and positive fractionation during the second part when 17% of U(VI) is remaining.



Figure 4.1 Concentration of U(VI) over time in U(VI) reduction experiments with magnetite as a reductant with 200  $\mu$ M U initial concentration (a) and 80  $\mu$ M U initial concentration (c) in duplicates.  $\delta$ 238U values of remaining U(VI) (solid phase extracted) plotted against the fraction of U(VI) reduced (C/C0) at 200  $\mu$ M U initial concentration (b) and 80  $\mu$ M U initial concentration (d) in duplicates.

To explore how the final speciation of U(IV) affects the isotopic fractionation, we set up an experiment where U(VI) was reduced by Fe<sub>3</sub>O<sub>4</sub> that was pre-sorbed with PO<sub>4</sub><sup>3-</sup>. As previously demonstrated<sup>9</sup>, the presence of PO<sub>4</sub><sup>3-</sup> on the surface of magnetite hinders the crystal growth of uraninite nanoparticles as it would occur during reduction with pure Fe<sub>3</sub>O<sub>4</sub>. Hence, U(IV) is immobilized as non-crystalline U(IV) (lacking the U-U pair coordination in the EXAFS). As shown in Figure 4.2a, the pre-treatment significantly affected also the reactivity of Fe<sub>3</sub>O<sub>4</sub> (Figure 4.2a) if compared to the original Fe<sub>3</sub>O<sub>4</sub> (Figure 4.2a and Figure 4.2c). Despite the fact that PO<sub>4</sub><sup>3-</sup> was pre-sorbed on Fe<sub>3</sub>O<sub>4</sub>, the reduction yield positive  $\delta^{238}$ U (Figure 4.2b). As in previous cases with pure magnetite, we observe no fractionation in the first phase of reaction and strong positive fractionation towards the end of the reaction. If shell-by-shell fit analysis of U L<sub>III</sub>-edge EXAFS will confirm that U(IV) is amorphous as in Veeramani et *al.*<sup>9</sup>, rather than UO<sub>2</sub> as expected after biotic reduction by magnetite, then this result will suggest that final speciation of reduction does not affect isotopic fractionation. (EXAFS analysis is pending).

U isotopic fractionation during U adsorption on maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) as a Fe(II)-free proxy for magnetite was investigated at two different U:Fe molar ratios: 0.016 and 0.028.  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> has the same structure and magnetic properties as magnetite, but, because it does not harbour Fe(II), U(VI) is not reduced. On the contrast to previous experiments, where total U and  $\delta^{238}$ U were measured after bicarbonate extraction, aqueous phase U was separated from the solids by filtration of the suspension through 0.22 µm PTFE filters (ThermoFisher, USA) without bicarbonate extraction for ICP-MS and MC ICP-MS measurements.

The rate of U adsorption on maghemite is slower than what was observed on magnetite (i.e., instantaneous adsorption of U on the minerals with no aqueous U left in solution) (Figure 4.1c). The highest  $\delta^{238}$ U (0.63±0.08‰) is recorded at low U:Fe loading after 240 hours from the beginning of the experiment when 5% of U was left in solution. At higher U:Fe molar ratio,  $\delta^{238}$ U fractionation is observed after 240 hours, but the extent of fractionation was lower than at 0.016 U:Fe (0.11±0.05‰). It has to be noted that

the equilibrium was not reached yet after 240 hours in the experiment with 0.016 U:Fe molar ratio. These findings are in agreement with previous studies showing similar fractionation during adsorption on goethite and ferrihydrite  $(0.39\pm0.04\%)^{17}$  and on birnessite  $(0.22\pm0.09\%)^{18}$ . Thus, U adsorption on maghemite exhibits the same direction of fractionation than U reduction by magnetite, but the extent of fractionation is smaller. As the isotopic signature of adsorption has a smaller magnitude than reduction, this may indicate that, after adsorption, reduction at the surface of magnetite is responsible for the extreme fractionation values observed in this study (i.e., ~5.5‰ at 0.016 U:Fe; (figure 4.1a)).



Figure 4.2 140  $\mu$ M U initial concentration (c).  $\delta^{238}$ U values plotted against the fraction of U(VI) adsorbed in experiments (C/C<sub>0</sub>) with 80  $\mu$ M U and 140  $\mu$ M U initial concentration (d).

Uranium reduction and isotopic fractionation were investigated in reduction experiment with  $Fe_3O_4$  in 'separate batches' to simultaneously monitor the speciation of U immobilized in the solids and the fractionation of U(VI) remaining in solution as a function of time.

As in the case of U adsorption on  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>, isotopic fractionation was measured on the fraction of U(VI) remaining in solution without bicarbonate extraction. As in previous experiments, fractionation of U in the aqueous phase exhibits positive values (0.11±0.06‰) (Table 4.2) already at 0.5 hours when ~80% of U was adsorbed.  $\delta^{238}$ U peaked at of 0.3±0.03‰ after 9 hours. Despite the fact that no intermediate time points were available, we report that  $\delta^{238}$ U did not change in the next 21 hours of reaction ( $\delta^{238}$ U equals to 0.3±0.03‰ at 30 hours). Hence, the extent of fractionation observed in this experiment was closer to the case of U adsorption on  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> than to U reduction with magnetite at same U:Fe molar ratio.

# 4.4.2 Average valence state of uranium

Figure 4.3 shows U L<sub>III</sub>-edge XANES spectra of the end products of U reduction and adsorption experiments by bulk XAS. Depending on the experiment, these spectra could be fitted with a combination of U(IV) as uraninite nanoparticles (UO<sub>2</sub>), U(V) and U(VI) as U<sub>3</sub>O<sub>8</sub> and U(VI) as uranyl adsorbed on ferrihydrite (Figure SI-1). The selection of the reference compounds is based on the results of the combinatorial analysis in Athena<sup>16</sup>. Thus, the fits presented below are those with the smallest  $X_r^2$  (Table SI-1). As the U<sub>3</sub>O<sub>8</sub> reference compound consists of 2 U(V) and 1 U(VI) both in the uranate coordination, the results of the LCF analysis (Table SI-3 and Table SI-4) are corrected accordingly, and the contribution of the three valence states is reported after this correction in Table 4.1 and Table 4.2. Note that the accuracy of XANES LCF is assumed to be ±10%<sup>19</sup>. The LCFs are reported in Figure 4.1 andFigure 4.2.

We report that independently of the U:Fe molar ratio and the type of magnetite used (w/o pre-sorbed  $PO_4$ ), the dominant valence state is U(IV), between 66 and 81% (Table 4.1). This is also confirmed by the edge position of XANES in these samples (17,170.8 eV- 17,171.2 eV) that is closed to the edge position of

87

our U(IV) reference standard (17,171 eV) (Figure SI-2). A minor fraction is found as U(VI), but the contribution is considered negligible because the quantification is close to the accuracy of the technique. Furthermore, LCF indicate that U(V) is also found in these samples. On the other hand, the proximity of the edge position of U(V) (17,171.3 eV) to U(IV) (17,171 eV) (Figure SI-2) is a limiting factor in resolving the three valence state at L<sub>III</sub> edge accurately. The presence of U(V) is supported by the results of the sensitivity analysis (Table SI-1) indicating 3-4 times lower  $X_r^2$  of the LCFs including U<sub>3</sub>O<sub>8</sub> as a reference compared to the fits without U<sub>3</sub>O<sub>8</sub>. Whether U(V) occurs at these conditions, needs to be confirmed by M4 edge HERFD-XANES experiments.

On the other hand, U(VI) is the dominant valence state when U is adsorbed on  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> (Table 4.1). In fact, the spectra exhibit the evident resonance feature at 17,190 eV which is the distinctive evidence of uranyl coordination environment (Figure 4.3).


Figure 4.3 Normalized bulk U L<sub>III</sub>-edge XANES spectra of selected samples reported with the correspondent U:Fe molar in the experiment (black line), their LCFs (red dashed line) using U(VI) adsorbed on ferrihydrite as standard for U(VI) (blue), biogenic UO<sub>2</sub> as standard for U(IV) (yellow) and U<sub>3</sub>O<sub>8</sub> as standard for U(V) and U(VI) (dark red). Fit results are reported in

Table 4.1 Results of bulk U L<sub>III</sub> edge XANES linear combination fit of U(VI) reduction in batches with Fe<sub>3</sub>O<sub>4</sub> or PO4 sorbed magnetite at various U concentration and U(VI) adsorption on  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>. (-) standard not required for the fit based on the X<sub>r</sub><sup>2</sup> (Table SI-1). Values in parenthesis are the uncertainties of the LCF from Athena<sup>16</sup>. The content of U(VI) and U(VI) is corrected based on the LCF in Table SI-3 as U3O8 reference compound consist of 2 U(V) and 1 U(VI)

sample name	U:Fe	U(VI)	U(VI)	U(V)	U(IV)	R factor
	[M:M]	uranyl [%]	uranate [%]	uranate [%]	[%]	
Fe <sub>3</sub> O <sub>4</sub> (200 μM)	0.04	-	8(1)	17(1)	75(1)	5.52E-04
Fe <sub>3</sub> O <sub>4</sub> (80 μM)	0.016	-	8(1)	23(1)	66(1)	3.84E-04
PO <sub>4</sub> -Fe <sub>3</sub> O <sub>4</sub> (80 μM)	0.016	-	11(1)	13(1)	81(1)	3.54E-04
PO4-Fe <sub>3</sub> O <sub>4</sub> (80 μM)	0.016	-	8(1)	17(1)	75(1)	3.69E-04
γ-Fe <sub>2</sub> O <sub>3</sub> (140 μM)	0.028	99(2)	0(2)	0(2)	-	6.67E-04
γ-Fe <sub>2</sub> O <sub>3</sub> (80 μM)	0.016	83 (1)	1(1)	3(1)	13(1)	1.64E-04

XANES LCF revealed that the batch of magnetite used in the second set of reduction experiments in 'separate-batches' has lower reactivity than the magnetite used in previous 'single-batch' experiments. In fact, despite the fact that U was rapidly adsorbed onto the surface of the mineral (Figure SI-3), the extent of reduction was limited. LCF analysis reports that U(VI) represents 73 and 69% of total U at 0.5 and 3 hours respectively. U(VI) decreases to 56% after 9 hours, but the reaction did not proceed further as U(VI) content did not change after 30 hours. The consumption of U(VI) is confirmed by the progressive disappearance of the 'uranyl shoulder' at 17,190 eV (Figure 4.2). The fits are completed with a mixture of U(V) and U(IV) (Table 4.2).

As discussed above, the occurrence of U(V) is supported by the sensitivity analysis in Table SI-2. The contribution of  $U_3O_8$  to the fits is visible in the region of the XANES from the white-line position to higher energy (Figure 4.4) that is significantly improved by the use  $U_3O_8$  as a reference when compared to the fits that do not include  $U_3O_8$  (Figure SI-4). The energy position of the XANES also supports the occurrence of U(V) as these samples (i.e.17,172.3-17,171.9 eV) are positioned between those of U(VI) (17,173 eV) and U(IV) (17,171 eV) references (Figure SI-2).

Despite the fact that the occurrence of U(V) needs to be confirmed, these results suggest that U(V) content increases from 16-17% in the early samples (0.5 and 3 hours) to 26 and 29% at 9 and 30 hours, respectively. M4 edge HERFD-XANES experiments are required to resolve the energy differences amongst the three valence states. On the other hand, these preliminary results suggest that U(V) may occur in this system and maybe be stable in Fe<sub>3</sub>O<sub>4</sub> as its contribution does not change from 9 to 30 hours.



Figure 4.4 Normalized bulk U L<sub>III</sub>-edge XANES spectra of selected samples (black line), their LCFs (red dashed line) using U(VI) adsorbed on ferrihydrite as standard for U(VI) (blue), biogenic UO<sub>2</sub> as standard for U(IV) (yellow) and U<sub>3</sub>O<sub>8</sub> as standard for U(V) and U(VI) (dark red). Fit results are reported in Table 4.2.

Table 4.2 U(VI) reduction in separate batches containing 5mM Fe as Fe3O4 and 80  $\mu$ M U; concentration of dissolved U over time, Isotopic fractionation of U ( $\delta^{238}$ U) remaining in the aqueous phase in per mil (‰) and results of the linear combination fit (LCF) analysis by bulk U LIII-edge XANES in percentages (%). The values in parenthesis are the uncertainties of the LCF from Athena<sup>16</sup>. The content of U(VI) and U(VI) is corrected based on the LCF in Table SI-4 as U<sub>3</sub>O<sub>8</sub> reference compound consist of 2 U(V) and 1 U(VI)

time	dissolved	C/C <sub>2</sub> [%] & <sup>238</sup> 11 [%_]		U(VI)	U(VI) U(VI)		U(V) U(IV)	
[h]	U [μM]	C/C0[/0]	0 0 [ /00 ]	[%]	[%]	[%]	[%]	R lactor
0.5	15.2	19.0%	0.11(0.06)	65(0.5)	8(0.5)	16(0.5)	10(0.5)	2.64E-05
3	10.6	13.3%	0.12(0.08)	60(0.6)	17(0.6)	17(0.6)	13(0.2)	4.19E-05
9	1.3	1.6%	0.3(0.03)	43(0.5)	26(0.4)	26(0.4)	17(0.2)	2.37E-05
30	0.3	0.4%	0.29(0.07)	42(0.5)	29(0.5)	29(0.5)	15(0.3)	3.18E-05

To summarize, the previous findings confirm that, during U(VI) reduction by synthetic magnetite, the lighter isotope is preferentially reduced leaving the unreacted U(VI) enriched in the heavy isotopes as indicated by positive  $\delta^{238}$ U values after bicarbonate extraction. These results are consistent with the previous findings from Stylo et *al.*<sup>3</sup>.

In addition, we observe that also the adsorption on maghemite in oxic solution preferentially removes the light isotopes. Despite the fact that the fractionation effect goes in the same direction of reduction, the extent of fractionation is smaller than during reduction. The extent of fractionation that we observe is consistent with the results from previous studies looking at U(VI) adsorption on other iron oxides and manganese oxides, such as goethite, ferrihydrite<sup>17</sup>, and manganese oxides<sup>18</sup>. Therefore, we conclude that, although adsorption does partially contribute to the total isotopic fractionation observed during reduction, this means that clearly reduction itself is responsible of the extreme fractionation behavior that we observed in this experiments where U is reduced by  $Fe_3O_4$  at 0:016 molar ratio (5.46-5.58‰) (Figure 4.1b).

Whilst we report that the highest  $^{238}$ U/ $^{235}$ U shift occurred during reduction at the highest U loading investigated in this study, we also report that more experiments are required to confirm this trend.

So far, the occurrence and persistence of U(V) have been demonstrated in previous works involving U(VI) reduction and incorporation of U(V) into the structure of magnetite<sup>20</sup> or on the surface of magnetite at acidic pH which again is likely to involve the incorporation of U(V) in the outer layer of Fe<sub>3</sub>O<sub>4</sub><sup>21</sup>. Therefore, this work presents the first evidence of persistent U(V) occurring at the surface of magnetite at neutral pH. On the other hand, because these findiings are based on L<sub>III</sub>-edge XAS data, that have a limited energy resolution to distinguish U(V) and U(VI), they need to be confirmed. In this regard, we suggest that future experiments should investigate U reduction at the same conditions using the M<sub>IV</sub> edge HERFD XANES, which is the most reliable technique available today for quantifying of U(V).

The XAS speciation of U on the solids from the experiments in 'separate batch' indicates that the content of U(V) and the isotopic fractionation increase concomitantly through time (Table 4.2). In fact, at 0.5 and 3 hours ( $\delta^{238}$ U =0.11-0.12‰ and 16-17% U(V) is detected) at 9 and 30 hours ( $\delta^{238}$ U=0.29-0.3‰ and 26-29% of U(V) is detected). More experiments at intermediate time points are required to confirm whether there is a correlation between the progressive enrichment of the light isotope in the products of reaction and the increment of U(V) that is detected on the surface of magnetite. These results are consistent with the hypothesis introduce above that the increment of U(V) is related to the isotopic fractionation observed.

As mentioned before, the occurrence of U(V) needs to be confirmed by further experiments at similar conditions. Future works should simultaneously and systematically probe the isotopic fractionation effect and the speciation of U on the surface of magnetite preferentially by the mean of  $M_{IV}$  edge HERFD XANES.

Despite the majority of the studies rely on the isotopic fractionation in the aqueous pool of U alone to infer the fractionation in the products, future works should simultaneously measure the <sup>238</sup>U/<sup>235</sup>U ratio in the pool of reactants and of the products at every time step throughout the entire duration of the reaction. This will allow to monitor the mass balance of the U isotopes at each time step and rule out the possibility that the isotopic fractionation is generated by experimental artifacts, for example during chemical extraction with bicarbonate and separation by filtration.

### 4.5 Acknowledgments

We are grateful to Katherine Morris and Sam Shaw for providing the U<sub>3</sub>O<sub>8</sub> standard.

### 4.6 References

(1) Weyer, S.; Anbar, A. D.; Gerdes, A.; Gordon, G. W.; Algeo, T. J.; Boyle, E. A. Natural Fractionation of 238U/235U. *Geochimica et Cosmochimica Acta* **2008**, *72* (2), 345–359. https://doi.org/10.1016/j.gca.2007.11.012.

(2) Basu, A.; Sanford, R. A.; Johnson, T. M.; Lundstrom, C. C.; Löffler, F. E. Uranium Isotopic Fractionation Factors during U(VI) Reduction by Bacterial Isolates. *Geochimica et Cosmochimica Acta* **2014**, *136*, 100–113. https://doi.org/10.1016/j.gca.2014.02.041.

 (3) Stylo, M.; Neubert, N.; Wang, Y.; Monga, N.; Romaniello, S. J.; Weyer, S.; Bernier-Latmani, R. Ura-nium Isotopes Fingerprint Biotic Reduction. *PNAS* 2015, *112* (18), 5619–5624. https://doi.org/10.1073/pnas.1421841112.

Bigeleisen, J. Temperature Dependence of the Isotope Chemistry of the Heavy Elements.
 Proceedings of the National Academy of Sciences 1996, 93 (18), 9393–9396.
 https://doi.org/10.1073/pnas.93.18.9393.

(5) Andersen, M. B.; Stirling, C. H.; Weyer, S. Uranium Isotope Fractionation. *Reviews in Mineralogy and Geochemistry* **2017**, *82* (1), 799–850. https://doi.org/10.2138/rmg.2017.82.19.

(6) Basu, A.; Brown, S. T.; Christensen, J. N.; DePaolo, D. J.; Reimus, P. W.; Heikoop, J. M.; Woldegabriel, G.; Simmons, A. M.; House, B. M.; Hartmann, M.; et al. Isotopic and Geochemical Tracers for U(VI) Reduction and U Mobility at an in Situ Recovery U Mine. *Environ. Sci. Technol.* **2015**, *49* (10), 5939–5947. https://doi.org/10.1021/acs.est.5b00701.

Brown, S. T.; Basu, A.; Christensen, J. N.; Reimus, P.; Heikoop, J.; Simmons, A.; Woldegabriel, G.;
 Maher, K.; Weaver, K.; Clay, J.; et al. Isotopic Evidence for Reductive Immobilization of Uranium Across a
 Roll-Front Mineral Deposit. *Environ. Sci. Technol.* 2016, 50 (12), 6189–6198.
 https://doi.org/10.1021/acs.est.6b00626.

 Bopp, C. J.; Lundstrom, C. C.; Johnson, T. M.; Sanford, R. A.; Long, P. E.; Williams, K. H. Uranium <sup>238</sup>
 U/ <sup>235</sup> U Isotope Ratios as Indicators of Reduction: Results from an in Situ Biostimulation Experiment at Rifle, Colorado, U.S.A. *Environmental Science & Technology* **2010**, *44* (15), 5927–5933. https://doi.org/10.1021/es100643v.

(9) Veeramani, H.; Alessi, D. S.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Sharp, J. O.; Dippon, U.; Kappler, A.; Bargar, J. R.; Bernier-Latmani, R. Products of Abiotic U(VI) Reduction by Biogenic Magnetite and Vivianite. *Geochimica et Cosmochimica Acta* **2011**, *75* (9), 2512–2528. https://doi.org/10.1016/j.gca.2011.02.024.

(10) Alessi, D. S.; Uster, B.; Veeramani, H.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Bargar, J. R.; Bernier-Latmani, R. Quantitative Separation of Monomeric U(IV) from UO2 in Products of U(VI) Reduction. *Environ. Sci. Technol.* 2012, *46* (11), 6150–6157. https://doi.org/10.1021/es204123z.

Wang, Y.; Morin, G.; Ona-Nguema, G.; Menguy, N.; Juillot, F.; Aubry, E.; Guyot, F.; Calas, G.; Brown,
 G. E. Arsenite Sorption at the Magnetite–Water Interface during Aqueous Precipitation of Magnetite: EX AFS Evidence for a New Arsenite Surface Complex. *Geochimica et Cosmochimica Acta* 2008, 72 (11), 2573–
 2586. https://doi.org/10.1016/j.gca.2008.03.011.

(12) Udo Schwertmann, R. M. C. *Iron Oxides in the Laboratory : Preparation and Characterization*; 2000.

(13) Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nature Methods* **2012**, *9* (7), 676–682. https://doi.org/10.1038/nmeth.2019.

Bosi, F.; Halenius, U.; Skogby, H. Crystal Chemistry of the Magnetite-Ulvospinel Series. *American Mineralogist* 2009, *94*, 181–189. https://doi.org/10.2138/am.2009.3002.

(15) Richter, S.; Eykens, R.; Kühn, H.; Aregbe, Y.; Verbruggen, A.; Weyer, S. New Average Values for the n(238U)/n(235U) Isotope Ratios of Natural Uranium Standards. *International Journal of Mass Spectrometry* **2010**, *295* (1), 94–97. https://doi.org/10.1016/j.ijms.2010.06.004.

(16) Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: Data Analysis for X-Ray Absorption Spectroscopy Using IFEFFIT. *J Synchrotron Radiat* **2005**, *12* (Pt 4), 537–541. https://doi.org/10.1107/S0909049505012719.

(17) Dang, D. H.; Novotnik, B.; Wang, W.; Georg, R. B.; Evans, R. D. Uranium Isotope Fractionation during Adsorption, (Co)Precipitation, and Biotic Reduction. *Environmental Science & Technology* 2016, *50* (23), 12695–12704. https://doi.org/10.1021/acs.est.6b01459.

(18) Brennecka, G. A.; Wasylenki, L. E.; Bargar, J. R.; Weyer, S.; Anbar, A. D. Uranium Isotope Fractionation during Adsorption to Mn-Oxyhydroxides. *Environmental Science & Technology* **2011**, *45* (4), 1370– 1375. https://doi.org/10.1021/es103061v.

(19) Kelly, S. D.; Kemner, K. M.; Carley, J.; Criddle, C.; Jardine, P. M.; Marsh, T. L.; Phillips, D.; Watson,
D.; Wu, W.-M. Speciation of Uranium in Sediments before and after In Situ Biostimulation. *Environ. Sci. Technol.* 2008, *42* (5), 1558–1564. https://doi.org/10.1021/es071764i.

Roberts, H. E.; Morris, K.; Law, G. T. W.; Mosselmans, J. F. W.; Bots, P.; Kvashnina, K.; Shaw, S. Uranium(V) Incorporation Mechanisms and Stability in Fe(II)/Fe(III) (Oxyhydr)Oxides. *Environ. Sci. Technol. Lett.* 2017, *4* (10), 421–426. https://doi.org/10.1021/acs.estlett.7b00348.

(21) Ilton, E. S.; Boily, J.-F.; Buck, E. C.; Skomurski, F. N.; Rosso, K. M.; Cahill, C. L.; Bargar, J. R.; Felmy, A. R. Influence of Dynamical Conditions on the Reduction of UVI at the Magnetite–Solution Interface. *Environ. Sci. Technol.* **2010**, *44* (1), 170–176. https://doi.org/10.1021/es9014597.

## Chapter 5 Conclusions

This last chapter summarizes the main findings of each chapter. Here, we also discuss the remaining knowledge gaps that need to be addressed.

Uranium (U) contamination of ground and surface waters is the legacy of decades of U mining and processing around the world. When high U concentrations in an aquifer pose an acute hazard for the ecosystem and human health, or when they surpass the regulatory threshold, action is required to contain the spread of the contaminant, remove it, or effectively immobilize it in the subsurface. Actions aimed to control the adverse effects and the spread of contamination elicit a substantial cost for industries, governments, and taxpayers.

In this regard, bioremediation of U via reductive precipitation has been investigated over the past few decades as a cost-effective strategy compared to other traditional techniques. Numerous field trials demonstrated that stimulating microbial processes in the subsurface effectively mediates the reduction of soluble U(VI) to sparingly soluble U(IV) which is then rapidly sequestered from solution and immobilized within the sediments. On the other hand, bioremediation mainly produces non-crystalline U(IV) (NCU4) species which were shown to be labile and more readily oxidized and re-mobilized than the crystalline uraninite. Thus, NCU4 is a less desirable end-product of U(VI) reduction and the overall effectiveness of reductive bioremediation is now under debate.

Previous studies have hypothesized that aging of NCU4 under anoxic condition may lead to the transformation of amorphous species to crystalline UO<sub>2</sub>. The implication of those findings are that, because UO<sub>2</sub> is more recalcitrant to oxidation, the U(IV) products would become more resistant to oxidation. Thus, transformation through aging will represent a beneficial process for bioremediation. Furthermore, bioremediation would become a reliable strategy for remediation when enough time is allowed for the transformation of NCU4 to UO<sub>2</sub>.

The objective of Chapter 2 was to investigate this hypothesis. For this purpose, we produced U(IV) in natural sediments from Rifle, CO (USA), via a series of column experiments to mimic the conditions of the natural environment in the field as in a bioremediation intervention. The metabolic activity of

microorganisms that naturally occur in these sediments was stimulated using a mixture of electron donors. When the bioreduction phase was completed, U(IV) speciation in the sediments was characterized by U  $L_{III}$  edge XAS.

Analysis of the EXAFS data confirmed that bioreduced U was immobilized in the sediments primarily as non-crystalline U(IV) complexed with C and P. Although the neighbor-elements invoked in the second shell of the shell-by-shell models are consistent with previous studies using natural sediments, the models presented a cumulative number of atoms that is too high to be physically possible. This represents a limitation for the study, and the EXAFS models presented in Chapter 2 are currently under revision. We are exploring the possibility to fit the second shell with a different configuration as proposed in the models from Bargar et *al.*<sup>1</sup> for bioreduced U(IV) in natural sediments from Rifle. This configuration consists of two separated subshells that are occupied by phosphorus at two different distances representing respectively bidentate and monodentate phosphorus bound to U. This configuration successfully models the EXAFS data from a sample in this study that was taken as an example. Thus, the same model will be applied to the entire set of the EXAFS data that are presented in Chapter 2.

The speciation of the bioreduced U(IV) in the sediments was periodically probed via XAS over a period of 12 months, under stable and controlled anoxic conditions. Even though the definitive EXAFS models are not available yet, the preliminary analysis presented in Chapter 2 shows that the speciation of U(IV) does not change over the period of aging under stable anoxic conditions. Previous work on NCU4 aging in natural sediments from the U contaminated site at Sellafield, UK, reported a progressive increment of the peak at 3.6Å that is generated by the U-U coordination path. Conversely, the EXAFS data in this study do not present this feature. Hence we conclude that UO<sub>2</sub> is not a relevant species in our system and no new UO<sub>2</sub> formed over the period of aging. To conclude, this result does not support the hypothesis that NCU4 ages to UO<sub>2</sub>. On the contrary, it indicates that, under the investigated conditions, NCU4 is a persistent

species in anoxic environments. Similarly, NCU4 is found persistent also in an organic-rich deposit, alpine meadows or in wetlands that formed decades ago. These findings suggest that it is likely that NCU4 produced during bioremediation persists in the subsurface if anoxic conditions are maintained. Therefore, if a remediated site is regularly exposed to events that bring oxygen into the reduced zone, it needs to be monitored because incoming oxidants are likely to remobilize reduced-U from the sediments into the aqueous phase.

After demonstrating that NCU4 species are persistent through aging, we challenged the stability of NCU4 in a series of oxidation experiments. Chapter 3 investigates the resistance of NCU4 species, harbored in bioreduced sediments, under conditions that mimic redox fluctuations in the environment from anoxic to oxidizing conditions. This work provides experimental evidence that NCU4 species are labile and they are rapidly oxidized and remobilized into the aqueous phase when they are exposed to oxygen.

In addition, we challenged a hypothesis proposed in a previous laboratory study suggesting that mackinawite (FeS), a typical by-product of sulfate-reducing bacteria that is often found associated with U(IV) in the environment, may accelerate the remobilization of NCU4 from the solids into the aqueous phase. To test this hypothesis, we assessed the rates of NCU4 oxidation in the presence of biogenic FeS produced concomitantly with NCU4 during the biostimulation of sediments in column-experiments. Indeed, the addition of sulfate into the artificial groundwater stimulate the growth of sulfate-reducing bacteria (SRB), producing sulfide as a metabolic by-product complexing with aqueous Fe<sup>2+</sup> and forming insoluble ironsulfide species (FeS).

The oxidation and remobilization of NCU4 in the presence of FeS is compared to the oxidation/remobilization rate in a control-experiment where SO<sub>4</sub><sup>2-</sup> was not added to the groundwater and no FeS was produced. We show that, under certain conditions (i.e., high dissolved oxygen (DO) concentration), the presence of FeS accelerates the oxidation and re-mobilization of NCU4. In addition, we propose a new oxidative

mechanism according to which, the oxidation and mobilization of NCU4 are partly driven by reactive oxygen species (ROS) that are generated when FeS is exposed to oxygen. In fact, we observed that hydrogen peroxide, interpreted as a proxy for the occurrence of ROS, was generated during the oxidation of the bioreduced sediments. This agrees with previous works which demonstrated that ROS are formed upon the exposure of reduced species of iron (i.e., FeS and other Fe<sup>2+</sup> species) to oxygen.

In addition, we observe that ROS production depends on DO concentration in the aquifer, and the contribution of ROS to NCU4 oxidation diminishes at low DO concentration. Furthermore, the role of FeS becomes negligible at low DO. At this condition the rates of NCU4 oxidation in the presence and absence of FeS are equals.

While Chapter 2 and 3 provide a further understanding of the stability of NCU4 as the main product of bioremediation, Chapter 4 explores a different aspect of U geochemistry that is the use of U isotopes as a marker for U reduction. Potentially, this is a convenient tool to monitor reduction processes in a bioreduced zone. In fact, after remediation, the aquifer has to be monitored to ensure that U remains immobilized in the sediments. Although this is traditionally achieved by measuring the concentration of U downstream the bio-stimulated zone, this method is affected by uncertainties linked to 'competing' processes that also scavenge U from solution (i.e., adsorption or coprecipitation). To avoid this, U isotopes could be possibly used to deconvolute the actual process that removes U from the aqueous phase. On the other hand, although isotopic fractionation has the potential to be applied as a marker for the reduction processes in the subsurface, its use is currently hindered by the limited understanding of the mechanism of fractionation. In fact, numerous studies demonstrated that there are a variety of abiotic and biotic processes that are capable of U isotopes fractionation and they are likely to co-occur simultaneously in nature. Therefore, a deeper understanding of these processes is warranted to confirm the potential use of U isotopes as a tracer for U reduction and to develop this tool further. In this regard, one of the knowledge gaps that remain to be investigated is the mechanism of isotopes fractionation during abiotic reduction. Chapter 4 aims to investigate the isotopic fractionation of U during abiotic reduction by magnetite (Fe<sub>3</sub>O<sub>4</sub>). Magnetite is a Fe<sup>2+</sup>-bearing mineral that is frequently found in the reduced zone in the aquifer.

Chapter 4 presents the results of a series of batch experiments using synthetic  $Fe_3O_4$  as a reductant at different U loading on the mineral, and synthetic maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) to assess the contribution of adsorption prior to reduction. This work confirmed that Fe3O4 preferentially reduces the 235U, as indicated in a previous study. This isotopic behavior is opposite than what predicted by the nuclear field shift effect during equilibrium fractionation and observed during biotic reduction. Therefore, it was inferred that a kinetic effect drives the U isotopes shifts during abiotic reduction by magnetite. We also report that the highest 238U/235U shifts occurred during reduction by magnetite at the highest U loading investigated suggesting that U loadings affect the extent of fractionation.

The control experiment using maghemite as a proxy for non-reactive magnetite revealed that adsorption fractionates U isotopes in the same direction as reduction, but the extent of fractionation is smaller. Therefore, the contribution of adsorption to fractionation is limited, and the reduction itself is responsible for the extreme fractionation behavior that is reported in this study.

In Chapter 4 U reduction has been investigated using two batches of magnetite with different reactivity; the first batch was capable of completely reduce U(VI) to U(IV), while the second batch was less reactive and a significant fraction of U(V) was detected as an intermediate product. This finding revealed that, under certain conditions, U(V) is persistent and stable species on the surface of the Fe<sub>3</sub>O<sub>4</sub>. On the other hand, it must be noted that this work characterized the speciation of U in the solid by U L<sub>III</sub> edge XANES, which has a limited energy-resolution to unequivocally distinguish the edge position of U(V) and U(VI).

Therefore, these findings need to be confirmed by XAS measurement at the  $M_{IV}$  edge with HERFD XANES experiments.

Until now, the occurrence of U(V) has been reported only in experiments involving the simultaneous reduction and coprecipitation of U into the structure of magnetite or in reduction experiment at acidic pH. Hence, if these results will be confirmed, they will represent the first evidence of stable U(V) at neutral pH on the surface of magnetite.

### 5.1 Outlook

The results presented in this thesis contribute to the collective understanding of the fate of NCU4 in the environment. These findings reveal that, although bioremediation effectively sequesters U(VI) from the aqueous phase in the short term, it may not be a reliable technique in the long term if the site is susceptible to redox variations. In fact, whilst NCU4 species immobilized within the remediated site are likely persistent under stable anoxic conditions, they remain vulnerable to variations in geochemical conditions and exposure to oxidants.

Furthermore, we show that FeS does not protect NCU4 from the oxidation by  $O_2$  as is the case of  $UO_2$ . Conversely, under certain conditions, FeS enhances the oxidation of NCU4 when a significant amount of  $O_2$  enters into the reduced zone.

Whilst bioremediation remains an attractive strategy to tackle U contamination in the subsurface, the suitability of the proposed treatment needs to be assessed on a site-specific basis. In fact, it appears that the stability of NCU4 depends on a variety of geochemical parameters that are likely to differ from site to site, (e.g., groundwater composition, mineralogy of the sediments, diffusion capacity in the subsurface and seasonal variations in geochemistry).

For example, with regards to this study we report that whilst NCU4 partially transforms to  $UO_2$  in the sediments from Sellafield, U.K. used by Newsome et *al.*<sup>3</sup>, no  $UO_2$  is found in the sediments investigated in this work and in Bargar et *al.*<sup>1</sup> under similar conditions of aging. Therefore, it is likely that the mechanism of NCU4 transformation to  $UO_2$  is affected by factors that are specific of Sellafield, UK, but that is absent at Rifle, CO. (e.g., groundwater composition or mineralogy of the sediments). The mechanism and the factors driving the transformation of NCU4 to  $UO_2$  at Sellafield remains unknown. However, it has been hypothesized that NCU4 forms when U(IV) complexes with ligands such as carbonates and phosphate that prevent the crystallization of  $UO_2$ .

Thus, one could speculate that, given the heterogeneity of the sediments, reduced mineral surfaces harboring low C and P are potential micro-environments where U(VI) is reduced to monomeric U(IV), but over time, desorption of these compounds might lead to local high U(IV) concentrations and low C/P concentrations that are favorable to allow the nucleation and growth of UO<sub>2</sub> crystals. If so, the occurrence of these micro-environments and their frequency in the sediments might play a role in the transformation of NCU4 to UO<sub>2</sub>.

We also report that stable conditions in the subsurface are likely to be regularly disrupted by seasonal watershed dynamics or by fortuitous meteorological events that occasionally bring oxygen into the reduced zone. Hence, bioreduced zones in the aquifer might undergo alternating periods of anoxia and oxygenation. For example, at the Old Rifle site, DO varies between a low concentration of <0.1 mg/L (fall/winter) and a maximal concentration of 1.5-2 mg/L (spring/summer). Whilst these periodical redox changes in the aquifer are responsible for partially remobilizing reduced species of U(IV) into the aqueous phase, their effect on the speciation of U(IV) in the long term has not been investigated yet. Future laboratory studies should monitor the fate of NCU4 through cycles of oxic and suboxic phases that mimic

the seasonal variations of DO in the groundwater and monitor the geochemistry and the speciation of U in the solids.

Another significant aspect that is currently poorly understood is the role of the coordination environment of the second shell in the stability of NCU4. In general, the reactivity of NCU4 is attributed to the lack of crystallinity which is the characteristic feature in all amorphous U(IV) species. On the other hand, it appears that the neighbors in the second shell of NCU4 can vary significantly depending on the investigated site or on the conditions of the system. For example, NCU4 is found complexed with C (monodentate carbonate, bidentate carbonate or organic matter), P (monodentate or bidentate) and Si in clay minerals. Future work should investigate how different U(IV)-ligands in bioreduced NCU4 affect the stability of U(IV) both during aging and during oxidation by oxygen.

Traditionally, the importance of ROS as oxidizing agents in the subsurface have been overlooked because ROS production has long been attributed to photochemical processes and subsurface systems are generally deficient in O<sub>2</sub>. However, the redox conditions in the subsurface environments are frequently perturbated by O<sub>2</sub> from both natural and anthropogenic processes, and numerous studies have recently revealed that significant amount of ROS is generated at the interface between oxic and anoxic environments, and ROS are likely to be relevant oxidants in the field. In this regard, although a previous study already hypothesized that ROS are capable of oxidizing U(IV), the findings of chapter 2 are the first direct evidence for this oxidative pathway. To conclude, a more accurate understanding of the dark production of ROS and NCU4 oxidation by ROS is a pivotal aspect to predict the stability of NCU4 in the environment. In this regard, a few critical gaps of knowledge remain to be addressed. Future research should aim to: deconvolute the mechanism of formation of ROS, identify the factors, other than DO concentration, that affect ROS production and define the specific oxidative pathway of U(IV). Chapter 2 and 3 focused on the stability of NCU4 as the dominant product of bioremediation under anoxic conditions in the long term (chapter 2) and its reactivity under oxidizing condition (chapter 3). Chapter 4 instead investigates the mechanism of U isotopes during abiotic reduction of magnetite. Although this represents a different aspect of U geochemistry, the future application of U isotopes fractionation in the field has a great potential as a tracer to monitor U reduction in the subsurface and, for example, at biore-mediated sites.

Chapter 4 presents the results of a series of experiments probing the isotopic fractionation of U during reduction by synthetic magnetite nanoparticles. On the contrary to biotic reduction, chapter 4 shows that  $Fe_3O_4$  preferentially reduces the <sup>235</sup>U. Furthermore, we reported that the loading of U on  $Fe_3O_4$  might affect the magnitude of isotopic fractionation. On the other hand, this correlation needs to be confirmed by additional experiments at the same conditions as in chapter 4 but at different U loading to support these results.

Furthermore, to address the hypothesis that U(V) is a transient intermediate species between U(VI) and U(IV), the occurrence of U(V) on magnetite and its role in the mechanism of isotopic fractionation needs to be investigated in experiments that combine  $M_{IV}$  edge HERFD XANES with accompanying measurements of isotopic signature.

Recent work demonstrated that isotopic fractionation also depends on the speciation of aqueous U(VI) and on the formation of calcium-carbonate complexes. This work suggests that initial speciation of U(VI) in the aqueous phase, which in turn affect the rate of reaction, has a significant impact on the isotopic signature. In fact, under a specific condition, abiotic reduction shows an isotopic behavior that is similar to biotic reduction. After this work, future experiments are required to make a step closer to the environment. A new experiment needs to be designed to replicate the geochemical conditions in the subsurface

environment better. Thus, the use of complex media with compositions that replicate natural groundwaters is urgent.

To conclude, the role of U(IV) speciation in isotopic fractionation has also been overlooked. To address this, reduction experiments need to investigate the isotopes fractionation needs in systems that generate multiple different U(IV) species, for example, NCU4 versus crystalline UO<sub>2</sub> nanoparticles. Although there is limited evidence to suggest that in pure cultures of *Shewanella oneidensis* that any difference is minimal, such investigations would benefit from a more comprehensive and systematic approach. Additionally, systems in which both U(VI) and U(IV) remain complexed in solution, e.g., by citrate, should be characterized, as this may enhance the ability of the system to reach isotopic equilibrium. Collectively, these results would provide mechanistic details which would enhance the robustness of U isotope signatures as tools for monitoring bioremediation.

# Annex 1 Supporting information Chpt. 2

### Speciation of sulfur

Sulfur XANES spectra (Figure SI 9) of the first layer from SRC2 and SRC4 are characterized by multiple oxidation states that were identified by comparing the energy position of the adsorption edges in model compounds used for the LCF analysis. The following oxidation states are clearly evident in the samples: S(-II) at 2,470.99-2,470.3 eV, S(0) at 2,472.2 eV and S(VI) at 2,482.6 eV. However, the dominant peak is positioned at 2,470.3 eV indicating that most of the S is present as S(-II). Thus, the best fits were obtained including mackinawite that was exposed sub-stoichiometric amounts of Fe(III). This FeS phase, referred as minimally oxidized mackinawite, exhibits a peak at 2470.3 eV, i.e. a peak shift in S XANES up to -0.6 eV compared to theoretical mackinawite <sup>1</sup> (Figure SI-11) (Table SI 7). The FeS model compound represents 91-95% of the total S in the system. The remaining fractions in S XANES are modeled by S(-I) as S associated with organics and a minor contribution of S(VI) (2-3%). Although the contribution of S(VI) in the fits is lower than the accuracy of the technique as reported in the literature (10%), it is considered as a relevant species as the peak is clearly distinguished in the spectra as discussed by Prietzel et *al.*<sup>2</sup>.

### Scanning transmission electron microscocopy

Sample was prepared by suspending the powder in ethanol by ultrasonication and drying a drop of the suspension on a carbon coated copper grid. STEM with energy dispersive spectroscopy (EDS) was used to obtain elemental composition maps and to perform comparative characterization of elemental content. In this study, an X-ray EDS system (Esprit/Quantax Bruker) in STEM mode in a FEI Tecnai Osiris microscope (200 kV X-FEG field emission gun, X-ray detector (Super-X) with 4 × 30 mm2 windowless SDD diodes and 0.9sr collection angle was applied. Quantitative EDS analysis was carried out using the Cliff-Lorimer standard-less method with thickness correction using K-series for all elements except uranium where M-series was used. The physical Bremsstrahlung background was calculated based on the sample composition. Some elements such as Cu contributing from the Cu grid were removed from quantification after the deconvolution procedure in the quantification process. Elemental concentrations in atomic % and net counts (signal above back-ground) were derived from deconvoluted line intensities within a 95% confidence level. The process time and

acquisition rates were adapted to get the most accurate data. A correction for specimen drift was applied during acquisition to improve elemental mapping accuracy.

ANIONS	mM
Ca2+	4
К+	0.3
Mg2+	3.94
Na+	7.52
CATIONS	
CI-	22.7
НСОЗ-	1
SO42-	14
рН	7.2

 Table SI- 1 Rifle artificial groundwater (RAGW)

Table SI- 2 Total amount of uranium leached from SRC2, SRC3, SRC4 before the start of the U(VI) bioreduction phase (121 days) and at the end of the experiment (407 days)

column	U leac	U leached	in 407 days	RABS/column	total U/column	
	(µmoles)	(mg)	(µmoles)	(mg)	(kg)	(mg)
SRC2	7.5	1.8	8.2	1.9	0.5	2.5
SRC3	6.0	1.4	6.5	1.5	0.5	2.4
SRC4	5.5	1.3	5.8	1.4	0.5	2.5

	SRC2	SRC3	SRC4
layer No.		U (ppm)	
1	461	549	479
2	96	71	61
3	80	27	28
4	71	22	16
5	14	19	14
6	18	18	8
7	9	9	8
8	9	6	5
total amount (mg)	55.93	51.42	45.89
Initial content of U in column prior to biostimulation (mg)	2.5	2.4	2.5

#### Table SI- 3 Uranium content per layer in SRC2, SRC3, SRC4.

Table SI- 4 Results of linear combination fit analysis of Fe K edge EXAFS spectra of RABS, SRC 4 and IRC6. The definition of R-factor and Chi-square are provided in SI Equation 1 and 2.

	RABS	SRC
model compounds	(%)	(%)
1.Illite	22	52
2.Biotite	23	19
3.Goethite	33	4
4.Hematite	20	0
5.Magnetite		
6.Mackinawite	2	25
7.Siderite		
R-factor (%)	1.53	2.08
Fe content (wt. %)	4.83	5.5

	S	RC4		SRC2				
model compounds	LCF results (%) (wt. %) LCF result				(%)	(wt. %)		
1. Mackinawite*	1	94.6	1.61	0.914	90.9	1.34		
2. Elemental sulfur	0 0		0.00	0.014	1.4	0.02		
3. S_in organics	0.03	2.8	0.05	0.046	4.6	0.07		
4. Sulfate	0.027	2.6	0.04	0.032	3.2	0.05		
sum	1.057		1.7	1.006		0.00		
R-factor			0.0152	0.0184				
Chi-square			1.5724			1.9053		
Reduced chi-square			0.0043	0.0052				
Sulfur content (wt. %)			1.7			1.5		

Table SI- 5 Results of linear combination fit analysis of S K edge XANES spectra of RABS bioreduced under sulfate-reducing. The definition of R-factor and Chi-square are provided in SI Equation 1 and 2. (\* Noel et al., 2017)<sup>1</sup>

	SI	RC2		SRC4			
model compounds	LCF results	(%)	(ppm)	LCF results	(%)	(ppm)	
1. U(IV) - NCU4	0.886	86	404.37	1	97	454.02	
2. U(VI) - Uranyl adsorbed on ferri- hydrite	0.146	14	66.63	0.033	3	14.98	
sum	1.032		471	1		469	
R-factor		·	0.0005	0.0032			
Chi-square			0.0282	0.1272			
Reduced chi-square			0.0002	0.0009			
content of Uranium (ppm)			471	469			

Table SI- 6 Results of linear combination fit analysis of U  $L_{III}$  edge XANES spectra of SRC2 and SRC4. The definitions of R-factor and Chi-squareare provided in SI Equation 1 and 2. Indicate k range

Table SI- 7 Results of the quantitative separation of U on the solids (%)

	U(\	/I)	NC	U4	UO2		
	(%)	s.d.	(%)	s.d.	(%)	s.d.	
SRC2	21%	1	70%	3	11%	5	
SRC4	17%	1	75%	3	8%	5	
SRC5	19%	2	70%	2	10%	5	

Table SI- 8 Results of shell-by-shell fitting procedure of U LIII edge EXAFS spectra of SRC 2 after bioreduction phase (no aging) and after an aging period of 4, 8 and 12 months at 1 and 10 mM HCO3 RAGW.

		12 n	12 mo. aged 1 mM HCO3					12 mo. aged 10 mM HCO3			
	shell	withou	t U-U	with	J-U	without	t U-U	with U	I-U		
U-Oax	CN	0.3	(1)	0.3	(2)	0.3	(1)	0.3			
	R	1.69	(3)	1.66	(1)	1.70	(3)	1.70	(3)		
	σ²	0.003	(3)	0.003	(4)	0.003	(4)	0.003	(3)		
	Eo	3.9	(16)	-1.7	(23)	4.0	(23)	3.9	(26)		
U-Oeq1	CN	0.9	**	0.8	**	0.9	**	0.9	**		
	R	2.39	*	2.356	*	2.40	*	2.404	*		
	σ²		*		*		*		*		
	Eo		*		*		*		*		
U-C	CN	5.2	(10)	5.7	(11)	4.7	(11)	4.7	(12)		
	R	2.94	*	2.94	*	2.94	*	2.94	*		
	σ²		*		*		*		*		
	Eo		*		*		*		*		
U-Oeq2	CN	6.8	**	6.9	**	6.8	**	6.8	(4)		
	R	2.32	(12)	2.31	(10)	2.32	(3)	2.32	(2)		
	σ²	0.013	(1)	0.014	(7)	0.013	(1)	0.013	(1)		
	Eo	-2.5	(29)	-3.1	(25)	-1.7	(36)	-1.356	(40)		
U-U	CN			1.1	(12)			0.5	(20)		
	R			3.87	*			3.88	*		
	σ²				*				*		
	Eo				*				*		
U-P	CN	3.6	(9)	4.3	(4)	6.4	(57)	6.6	(65)		
	R	3.59	-	3.595	-	3.59	-	3.59	-		
	σ²	0.008	(5)	0.008	(5)	0.014	(9)	0.015	(10)		
	Eo	1.8	(20)	1.2	(19)	1.5	(25)	1.597	(28)		
Chi-squar	e	4.3	32E+06	2.96E+06		1.7	3E+06	1.70E+06			
Reduced	chi Square	8.2	25E+05	1.2	26E+07	4.0	4.09E+05		5.26E+05		
R-factor			0.020		0.014		0.012		0.012		

Note: EXAFS fitting parameters include coordination number (CN), interatomic distances (R(Å)), Debye Waller factor (σ<sup>2</sup>(Å)) and energy shift ΔE<sub>0</sub> (eV). The uncertainties of the fit are given in parenthesis for the last significant figure. (\*) parameters linked to the one above in the table. (-) fixed parameter. (\*\*) the total number of oxygen atoms is constrained to 8. Each model contain one MS path for the uranyl moiety. The accuracy of the fit is evaluated by R<sub>f</sub> as defined in equation SI-1.

	U(VI)ª	U(IV)⁵	R <sub>f</sub>
	+/-10% (%)	+/-10% (%)	(10 <sup>-3</sup> )
initial	14.0	86	0.5
4 mo. 1 mM HCO3	4	96	2.1
8 mo. 1 mM HCO3	2.5	98.0	7.8
12 mo. 1 mM HCO3	2	98	1.7
4 mo. 10 mM HCO3	3	97	3.9
8 mo. 10 mM HCO3	3	97	5.3
12 mo. 10 mM HCO3	3	97	2.9

Table SI- 9 Results of linear combination fit analysis of U L<sub>III</sub> edge EXANES spectra of the initial sediment (SRC2) and agesd sediment at 1 mM and 10 mM bicarbonate concentration. The XANES LCF components are bioreduced non-crystalline U(IV) (NCU4) and Uranyl adsorbed on ferrihydrite (Figure SI-12). XANES LCF parameters are given in percentage of the total uranium in the sample. R-factor is defined as in Equation SI-1.

Spectrum	U	0	Mg	Al	Si	Р	S	К	Ca	Ti	V	Mn	Fe	Ni	Rb
[at.%]															
180920-	0.01	24.33	1.43	2.57	6.9	0.04	34.07	0.4	1.26	0.05	0.06	0.08	28.74	0.06	0
12m-a-03															
180920-	0.02	55.91	0.98	4.71	22.26	0.51	6.66	0.27	1.68	0.38	0.1	0.03	6.46	0.03	0
12m-a-															
04-1															
180920-	0.77	59.11	1.73	6.08	16.07	1.98	3.36	1.52	4.09	0.05	0.1	0.01	5	0.07	0.06
12m-a-															
04-2															
180920-	0.09	47.65	1.8	5.85	5.02	3.4	13.77	0.21	5.07	0.44	0.58	0.09	15.95	0.06	0.02
12m-a-05															
180920-	0.99	58.74	1.16	3.57	9.17	0.32	3.2	0.12	2.03	0.37	0.09	0.11	19.88	0.19	0.08
12m-a-06															
Mean	0.37	49.15	1.42	4.56	11.88	1.25	12.21	0.51	2.82	0.26	0.19	0.06	15.21	0.08	0.03
value:															
Sigma:	0.47	14.62	0.35	1.49	7.15	1.42	12.95	0.58	1.66	0.19	0.22	0.04	9.83	0.06	0.03
Sigma	0.21	6.54	0.16	0.67	3.2	0.63	5.79	0.26	0.74	0.09	0.1	0.02	4.39	0.03	0.02
mean:															

Table SI- 10 EDS data STEM picture 180920-12m-a-03, 041, 042, 05 and 06  $\,$ 

Spectrum	0	Mg	AI	Si	Р	S	K	Ca	Ti	V	Mn	Fe	Ni	Cu	Zr	Pb	U
Figure SI-	24.	1.3	2.5	6.8	0.0	34.	0.4	1.2	0.0	0.0	0.0	28.	0.0	0.0	0.0	0.0	0.0
3	31	8	4	9	3	15	0	6	4	5	7	81	6	0	0	0	1
Figure SI-	56.	0.9	4.7	22.	0.5	6.4	0.1	1.6	0.3	0.1	0.0	6.1	0.0	0.0	0.0	0.0	0.0
4.1	04	4	5	83	1	7	2	3	9	0	3	4	3	0	0	0	2
Figure SI-	59.	1.7	6.1	16.	1.9	3.3	1.5	4.0	0.0	0.1	0.0	5.0	0.0	0.0	0.0	0.0	0.8
4.2	10	5	0	09	9	6	2	7	4	0	1	0	7	0	0	0	0
Figure SI-	47.	1.7	5.8	5.0	3.4	13.	0.2	5.0	0.4	0.5	0.0	15.	0.0	0.0	0.0	0.0	0.1
5	67	8	4	1	0	77	1	7	4	8	9	96	6	0	0	0	0
Figure SI-	60.	0.6	0.9	6.1	0.2	1.9	0.0	2.0	0.2	0.0	0.1	23.	0.3	0.0	0.0	0.0	2.8
6	53	4	2	5	1	2	0	8	9	6	7	90	2	0	0	0	1
Mean	49.	1.3	4.0	11.	1.2	11.	0.4	2.8	0.2	0.1	0.0	15.	0.1	0.0	0.0	0.0	0.7
value:	53	0	3	39	3	94	5	2	4	8	7	96	1	0	0	0	5
Sigma:	14.	0.5	2.2	7.7	1.4	13.	0.6	1.6	0.1	0.2	0.0	10.	0.1	0.0	0.0	0.0	1.2
	95	0	3	7	4	23	1	6	9	3	6	54	2	0	0	0	0
Sigma	6.6	0.2	1.0	3.4	0.6	5.9	0.2	0.7	0.0	0.1	0.0	4.7	0.0	0.0	0.0	0.0	0.5
mean:	9	2	0	7	4	2	7	4	9	0	3	1	5	0	0	0	4

Table SI- 11 EDS measurements of biostimulated sediments after aging for a period of 12 months in RAGW at 1 mM HCO<sub>3</sub>.



Figure SI-1 XANES (left) and EXAFS (right) spectra of reference compound used for linear combination fit analysis of U K edge



Figure SI- 2 XANES spectra of reference compound utilized for linear combination fit analysis of S K edge XANES



Figure SI- 3 XANES (left) and EXAFS (right) spectra of reference compounds used for linear combination fit analysis of Fe K edge



Figure SI- 4 Iron concentration in the effluents over time of SRC2, SRC3, SRC4



Figure SI- 5  ${\rm Iron(II)}$  concentration in the effluents over time of SRC2, SRC3, SRC4



Figure SI- 6 Sulfate concentration in the effluents over time of SRC2, SRC3, SRC 4



Figure SI- 7 XRF measurements of the iron content per layer in SRC and IRC columns. The dotted line represents the average concentration of Fe in RABS



Figure SI- 8 Sulfur K edge XANES of the RABS bioreduced under sulfate-reducing conditions column 2 and 4 (SRC2 and SRC4)



Figure SI- 9 XRF measurements of the sulfur content per layer in SRC columns. The dotted line represents the concentration of S in RABS.



Figure SI- 10 Iron K edge EXAFS of RABS before and after the bioreduction phase under sulfate-reducing conditions (SRC) (col. no. 4)


Figure SI- 11 XRF measurements of the uranium content per layer in SRC column 2, 3 and 4 (SRC2, SRC3, SRC4



Figure SI- 12 Uranium  $L_{\rm III}$  edge XANES of the SRC2 and SRC4 bioreduced under sulfate-reducing conditions



Figure SI- 13 Fourier transforms of uranium L<sub>III</sub> edge EXAFS spectra (full lines) and shell-by-shell fits (black dotted lines) of SRC2 and SRC4.



Figure SI- 14 Uranium L<sub>III</sub> edge EXAFS spectra (full lines) and shell-by-shell fits (black dotted lines) of SRC columns no. 2 and 4



Figure SI- 15 Result of Shell-by-shell fit analysis of 12 months aged samples at high bicarbonate (a) and low bicarbonate (b). Fits a1 and b1 contains the contribution of U-U path with CN 1.2 in a1 and 0.5 in b1 and fits a2 and b2 do not. The goodness of the fits is evaluated on the R-factor ( $R_f$ ) and the Reduced Chi-squared ( $X^2_r$ ) as defined in Equation SI-1 and Equation SI-2.



 $Figure \ SI-16 \ STEM \ 180920\_12m\_a\_03 \ Imaging \ of \ bioreduced \ sediment \ after \ 12 \ months \ aging \ in \ RAGW \ at \ 1 \ mM \ HCO_3$ 



Figure SI- 17 STEM image of bioreduced sediment after 12 months aging in RAGW at 1 mM  $\ensuremath{\mathsf{HCO}_3}$ 



Figure SI- 18 STEM image of bioreduced sediment after 12 months aging in RAGW at 1 mM  $\ensuremath{\mathsf{HCO}_3}$ 



Figure SI- 19 STEM image of bioreduced sediment after 12 months aging in RAGW at 1 mM  $\rm HCO_3$ 



Figure SI- 20 pH in the effluents over time of SRC2, SRC3, SRC4



Figure SI- 21 XRF measurements of the total organic carbon content per layer in SRC and IRC columns

$$R_factor = \frac{\sum (X(k)_{experimental \ data} - X(k)_{fit})^2}{\sum (X_{experimental \ data})^2}$$

Equation SI-1 definition of R-factor

$$X_r^2 = \frac{N_{ind}}{vn} \sum_{i=1}^n \frac{(k^3 X(k)_{experimental \ data,i} - k^3 X(k)_{fit,i})^2}{\varepsilon_i^2}$$

Equation SI-2 Definition of reduced Chi-square Xr<sup>2</sup>

# Annex 2 Supporting information Chpt. 3

## Characterization of IRC sediment

A detailed discussion of the bioreduction of RABS in column-experiments under sulfate reducing conditions (SRC) is presented elsewhere (Manuscript in preparation). LCF of X-ray near edge absorption spectroscopy (XANES) at the U LIII edge from SRC columns 2 (SRC2), 4 (SRC4) and IRC column 6 (IRC6) (Figure SI 3) revealed that all samples were mostly dominated by tetravalent uranium. The highest content of U(VI) was found in SRC2 (14%), while SRC4 and IRC6 contained less than 10% (Table SI 2). Shell-by-shell fit analysis of EXAFS (Figure SI-5 and Table SI-3) confirmed that the dominant frequency is generated by 5.3-7.1 U-O scattering paths at ~2.32Å that is consistent with U<sup>4+</sup> coordinated to oxygen. The minor frequency generated by U-Oax path at ~1.8 Å accounts for a fraction of U(VI) that is also found in XANES LCF. The second shell was modeled with bidentate C, and monodentate P in agreement with the models of microbially reduced NCU4 reported in <sup>2–4</sup>.

## The effect of bicarbonate concentration in RAGW

The effect of bicarbonate concentration on the dissolution of uranium in flow through oxidation experiment is transient under under oxic conditions. In fact, high bicarbonate concentration dissolve U faster within the first 200 hours, but the effect disappeared in the last 100 hours. At the end of the experiment ~1100 nmoles of U were dissolved both at high and low bicarbonate concentration after 300 hours. On the contrary, under suboxic conditions, the effect of bicarbonate concentration in RAGW persisted throughout the entire duration of the experiment. Thus, at the end of the experiment, ~100 µmoles of U are dissolved more at 10 mM HCO3 than at 1 mM HCO3. 580.6-566.9 µmoles are leached at high bicarbonate respectively in SRC and IRC, while 470.5-462.2 µmoles are leached at low bicarbonate concentration in SRC and IRC.

Regardless of the type of sediments and DO concentration in RAGW, U speciation by chemical extraction of the solids indicated that adsorbed U(VI) occurred in the sediments. Adsorbed U(IV) increased in the first 3 hours of oxidation (being ~0% in the initial solids) and then slowly decreased during the entire duration of the experiment (Error! Reference source not found. and Error! Reference source not found.). Under oxic

conditions, adsorbed U(VI) in SRC decreased from 42.7-39.5% at 3 hours to 16.5-23.2% at 15 hours. Adsorbed U(VI) in IRC remains stable, and it ranged between 58.9 and 51.7%. When SOD and CAT were added to the sediments, adsorbed U(VI) increased to 81.12 % at three hours and decreased to only 77% at 15 hours. On average adsorbed U(VI) was higher under suboxic conditions than U(VI) under oxic conditions, but the extent of U(VI) dissolution was lower. 23% of U(VI) was dissolved in SRC (i.e., from 52.2-50.8% at 3 hours to 24.1-27.9% after 15 hours of oxidation). In IRC adsorbed U(VI) decreased from 76.3-82.3% at 3 hours to 68.2-72.5% at 15 hours.

As well as in previous works that quantified U speciation in Rifle sediments by bicarbonate extraction <sup>5–8</sup>, the fraction of recalcitrant U recovered by digestion in aqua regia (3:1 volume ratio of concentrated HCl and HNO<sub>3</sub>), was reported as crystalline UO<sub>2</sub> species. The content of UO<sub>2</sub> in SRC (11.16%) and IRC (14.53%) was similar. Furthermore, regardless of the DO concentration and the type of the sediments, the content of UO<sub>2</sub> remained constant over the time (**Error! Reference source not found.**) and **Error! Reference source not found.** and **Error! Reference source not found.**) confirming that this species was less reactive than NCU4 and it was hardly consumed by oxidation even at 8.56 mg/L DO.

## Kinetic modeling

The rate of U(VI) dissolution as analyzed in the batch oxidation experiments by interpreting the rate of soluble U(VI) production as a first-order process with respect to the total amount of U(IV). By constraining the amount of U as a constant in the system, the concentration of soluble U was a function of time (equation SI 1), as derived by Cerrato et *al.*<sup>9</sup>, with *k* being the first-rate constant (s<sup>-1</sup>). As the sediments were prewashed in 50 mM anoxic bicarbonate to remove the unreacted U(VI) fraction and the fraction of UO<sub>2</sub> remained constant over time; it was assumed that NCU4 was the only pool of uranium being oxidized and dissolved. Equation 1 was optimized via the optimization procedure of Solver in Excel by minimizing the sum of the squares of the differences between the model output and the experimental data. The goodness of the fits is estimated by calculation of the Chi-square factor (equation SI 2). U dissolution rate is higher in oxic (2.6E-05 mol•s<sup>-1</sup> in SRC and 9.6E-06 mol•s<sup>-1</sup> in IRC) than in suboxic experiments (1.15E-05 mol•s<sup>-1</sup> in SRC and 4.13E-06 mol•s<sup>-1</sup>

in IRC). The rate of NCU4 oxidative dissolution in the current study (i.e., 2.6E-05 mol•s<sup>-1</sup> in SRC and 9.6E-06 mol•s<sup>-1</sup> in IRC) is lower than in a similar experiment (2.364E-04 mol•mole<sup>-1</sup>•s<sup>-1</sup>) that investigated oxidative dissolution of pure NCU4 under oxic conditions <sup>9</sup>. This difference is attributable, at least partly, to the adsorption of U(VI) to sediment minerals in our system. In fact, this will be demonstrated upon quantification of total U(VI) in the system (adsorbed and aqueous) (**Error! Reference source not found.** and **Error! Reference source not found.**).

ANIONS	mM
Ca2+	4
К+	0.3
Mg2+	3.94
Na+	7.52
CATIONS	
CI-	22.7
НСОЗ-	1
SO42-	14
рН	7.2

Table SI- 12 Rifle artificial groundwater (RAGW)

	SRC	col. No. 2	2	SRC	col. No. 4	ļ	IRC col. No. 6			
model compounds	LCF results	(%)	(ppm)	LCF results	(%)	(ppm)	LCF results	(%)	(ppm)	
1. U(IV) - NCU4	0.886	85.85	404.37	1	96.81	454.02	1	97.28	464.01	
2. U(VI) - Uranyl andsorbed on Fer- rihydrite	0.146	14.15	66.63	0.033	3.19	14.98	0.028	2.72	12.99	
sum	1.032		471	1		469	1.028		477	
R-factor			0.0007			0.0032			0.0065	
Chi-square			0.0282			0.1272			0.2564	
Reduced chi-square			0.0002			0.0009		0.001		
content of Uranium (ppm)			471	469			477			

Table SI- 13 Results of linear combination fit analysis of U L<sub>III</sub> edge XANES spectra of SRC columns no. 2 and 4 and IRC column no. 6

shell		SRC2		SRC4	4	IRC	5
U-Oax	CN	0.6	(10)	0.2	(1)	0.3	(1)
	R	1.76	(2)	1.70	(6)	1.71	(6)
	σ²	0.003	-	0.003	(1)	0.003	(1)
	Eo	6.4	(3)	3.0	(10)	-2.8	(10)
U-Oeq1	CN	1.8	**	0.5	**	0.9	**
	R	2.46	*	2.43	*	2.41	*
	σ²		*		*		*
	Eo		*		*		*
U-C	CN	2.0	(8)	5.0	(12)	4.4	(10)
	R	2.94	-	2.94	-	2.94	-
	σ²		*		*		*
	Eo		*		*		*
U-Oeq2	CN	5.9	**	7.3	**	6.5	**
	R	2.43	(1)	2.32	(3)	2.31	(2)
	σ²	0.009	(1)	0.014	(1)	0.012	(1)
	Eo	-0.2	(14)	-3.4	(39)	-0.1	(19)
U-P	CN	3.7	(6)	7.0	(12)	5.2	(9)
	R	3.59	-	3.59	-	3.56	-
	σ²	0.008	(3)	0.017	(3)	0.021	(4)
	Eo	0.3	(28)	0.5	(23)	-3.9	(38)
R-factor		9.38E-(	03	2.22E-	02	1.33E-	02

Table SI- 14 Results of shell-by-shell fitting procedure of U L<sub>III</sub> edge EXAFS spectra from SRC columns 2 and 4 and IRC column no. 6

Notes: EXAFS fitting parameters include coordination number (CN), interatomic distances (R(Å)), Debye-Waller factor ( $\sigma^2$ (Å)) and energy shift  $\Delta E_0$  (eV). The uncertainties of the fit are given in parenthesis for the last significant figure. (\*) parameters linked to the one above in the table. (-) fixed parameter. (\*\*) the total number of oxygen atoms are constrained to 8. Each model contains one MS path for the uranyl moiety. The accuracy of the fit is evaluated by R<sub>f</sub> as defined in equation SI-1.

	RABS	SRC	IRC
model compounds	(%)	(%)	(%)
1.Illite	22	52	29
2.Biotite	23	19	30
3.Goethite	33	4	14
4.Hematite	20	0	19
5.Magnetite			5
6.Mackinawite	2	25	
7.Siderite			2
R-factor (%)	1.53	2.08	1.63
Fe content (wt. %)	4.83	5.5	4.5

Table SI- 15 Results of linear combination fit analysis of Fe K edge EXAFS spectra of SRC columns no. 2 and 4 and IRC column no. 6

Table SI- 16 Results of the quantitative separation of U in the sediment post-reduction

	total		U6		NCU4		UO2		
	(ppm)	s.d.	(ppm)	s.d.	(ppm)	s.d.	(ppm)	s.d.	
SRC2	471	21.6	78.3	5.7	342.7	11.6	49.9	27.5	
SRC4	485	24.2	81.6	9.7	363.8	17.8	39.6	32.3	
IRC	444	14.3	56.6	11.2	318.9	18.1	69.0	21.2	

	nmoles															
							SOD&CAT									
tim e	tota	IJ	U(\	/I)	NC	U4	UC	)2	total U U(VI) NCU4 UO2						02	
(hr	me	s.d	mea	s.d	mea	s.d	mea	s.d	me	s.d	mea	s.d	mea	s.d		s.d
)	an	•	n	•	n	•	n	•	an	•	n	•	n	•		•
0	516.	20	7 9	15.	458.	26.	10 0	30.	516.	20	7 8	15.	458.	26.	49.	30.
U	3	20	7.0	3	6	2	49.9	9	3	20	7.0	3	6	2	9	9
2	441.	14.	322.	18.	75.2	29.	12 6	25.	452.	17.	203.	23.	193.	33.	FC	27.
5	3	2	5	5	75.2	3	45.0	1	9	4	5	9	4	5	50	1
c	100	17.	396.	25	F1 2	41.	20.1	34.	437.	15.	269.	17.	112.	29.	55.	28.
0	480	3	7	25	51.2	8	38.1	1	2	5	6	1	2	9	4	3
15	464.	10	421.	24.	2.0	39.	10.0	26.	444.	15.	346.	17	C1 0	28.	36.	27.
15	8	12	3	8	2.8	7	40.6	9	6	9	4	1/	61.9	2	3	1

Table SI- 6 Results of HCO3 extraction after oxidation in batch experiments under oxic condition in SRC, data are in nmoles.

Table SI- 17 Results of HCO3 extraction after oxidation in batch experiments under oxic condition in IRC, data are in nmoles.

	nmoles															
									SOD&CAT							
tim e	tota	IU	U()	/I)	NCU4		UC	)2	total U U(VI)		/I)		NCU4		UO 2	
(hr	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d
)	an		an	•	an		an	•	an		an		an		an	•
•	428.	15.	15.2	12.	368.	23.	45.2	42.	428.	15.	15.2	12.	368.	23.	45.2	26.
U	7	4	15.2	2	3	5	45.Z	2	7	4	15.2	2	3	5	45.Z	7
2	385.	16.	118.	18.	210.	30.	EC /	29.	410.	39.	101.	22.	253.	32.		48.
5	8	8	8	5	6	9	50.4	1	8	2	1	5	2	2	50.5	8
<u> </u>	396.	12.	174.	17.	171.	33.	F0 C	38.	391.	15.	135.	19.	104	29.	C1 7	25.
6	5	1	1	9	7	9	50.6	1	1	8	5	9	194	7	61.7	6
15	411.	7 2	258.	20.	00.0	25.	52.0	12.	437.	31.	245	20.	129.	31.	c2 7	42.
15	9	1.2	2	1	99.8	7	53.9	9	9	1	245	1	1	3	63.7	4

	nmoles															
					SOD & CAT											
tim e	total U U(VI) NCU4 UO2				tota	IU	U(VI)		NC	U4	UO2					
(hr	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d
)	an		an	•	an	•	an	•	an	•	an	•	an	•	an	•
0	516.	20	7.8	15.	458.	26.	<u>1</u> 9 9	30.	516.	20	78	15.	458.	26.	<u>19 9</u>	30.
	3	20	7.0	3	6	2	45.5	9	3	20	7.0	3	6	2	45.5	9
2	506.	24.	198.	24.	263.	35.	115	35.	491.	11.	173.	13.	278.	31.	20.0	29.
3	3	2	2	5	6	3	44.5	1	9	4	8	9	2	5	39.9	1
c	500.	11.	277.	17	164.	29.	гоо	24.	526.	0.5	227	27.	242.	35.	F.C. A	18.
O	7	9	5	1/	4	2	50.0	1	2	9.5	227	1	8	9	50.4	3
15	504.	10	290.	14.	158.	29.	<b>FF</b> 4	30.	492.	17.	277.	17	179.	26.	25.0	27.
15	1	10	8	8	2	7	55.I	9	6	9	3	1/	7	2	35.0	1

Table SI- 18 Results of HCO3 extraction after oxidation in batch experiments under suboxic condition in SRC, data are in nmoles.

Table SI- 19 Results of HCO3 extraction after oxidation in batch experiments under suboxic condition in IRC, data are in nmoles.

	nmoles															
									SOD&CAT							
tim e	tota	IU	U(\	/I)	NCU4		UC	)2	total U U(VI)		/I)	NC	U4	UC	)2	
(hr	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d	me	s.d
)	an	•	an	•	an	•	an	•	an	•	an	•	an	•	an	•
0	428.	15.	15.2	12.	368.	23.	15 2	26.	428.	15.	15.2	12.	368.	23.	15.2	26.
U	7	4	15.2	2	3	5	45.Z	7	7	4	15.2	2	3	5	45.Z	7
2	379.	16.	00 6	14.	240.	27.	40 F	29.	387.	19.	04 5	18.	256.	30.	1C F	30.
5	8	8	98.0	7	7	1	40.5	1	2	2	84.5	5	2	2	40.5	8
	360.	13.	150.	12.	159.	23.	50.0	24.	336.	18.	145.	13.	120	28.	F4 7	33.
6	4	1	2	9	6	9	50.6	1	1	9	4	5	139	2	51.7	6
15	409.	17.	206.	14.	142.	23.	С1 Г	26.	415.	26.	198.	11.	154.	24.	c2 7	39.
15	8	2	1	1	2	7	01.5	9	9	2	1	1	1	1	63.7	2

		(μmoles)										
		SR	с		IRC							
time (hr)	total U	U(VI)	NCU4	UO2	total U	U(VI)	NCU4	UO2				
0	1.66	0.02	1.44	0.21	1.62	0.000	1.34	0.29				
21	1.53	0.83	0.45	0.24	1.70	0.97	0.53	0.20				
68	1.71	1.013	0.44	0.25	1.70	1.10	0.40	0.20				
296	1.55	1.24	0.07	0.25	1.42	1.21	0.08	0.16				

Table SI- 20 Results of HCO3 extraction after oxidation in flow-through experiments under oxic conditon in SRC and IRC under high bicarbonate

Table SI- 21 Results of HCO3 extraction after oxidation in flow-through experiments under suboxic conditon in SRC and IRC

				(μm	oles)			
		SR	с			IR	C	
time (hr)	total U	U(VI)	NCU4	UO2	total U	NCU4	UO2	
0	1.66	0.02	1.44	0.21	1.62	0.000	1.34	0.28
296	1.76	1.01	0.48	0.27	1.66	0.90	0.50	0.25

#### Table SI- 22 Oxygen and solids content in batch and oxic flow-through oxic reactor

	unit	batch oxic	batch suboxic	flowthrough oxic
(02)	(mg/L)	8.56	1.07	8.56
solids	(mg)	300	300	1000
RAGW	(mL)	30	30	12.5
O2/solid	(mgO2/gsolids)	0.856	0.107	0.107

	SRC col. No	<b>b.</b> 4	21h		68 h		296 h	
model compounds	LCF results	(%)	LCF results	(%)	LCF results	(%)	LCF results	(%)
1. Fe in clays	0.468	41.75	0.411	34.42	0.457	36.74	0.330	23.52
2. Ferric oxides	0.390	34.79	0.645	54.02	0.643	51.69	0.842	60.01
3. Magnetite	-	-	-	-	0.088	7.07	0.197	14.04
4. Siderite	0.047	4.19	0.054	4.52	-	-	-	-
5. Mackinawite	0.216	19.27	0.084	7.04	0.056	4.50	0.034	2.42
sum	1.121		1.194		1.244		1.403	
R-factor		0.0126		0.0068		0.0051		0.0096
Chi-square		0.3123		0.2016		0.1357		0.2275
Reduced chi-square		0.0020		0.0016		0.0010		0.0018

## Table SI- 23 Results of LCF analysis of Fe K-edge XANES spectra of SRC (a) and IRC (b) sediments before and after oxidation in batch experiments under oxic conditions in flowing through CSTR reactor at 10 mM HCO3.

Table SI- 24 Results of LCF analysis of Fe K-edge XANES spectra of IRC sediments before and after oxidation in batch experiments under oxic conditions in flowing through CSTR reactor at 10 mM HCO3

	IRC col.	6	21 h		68 h		296 h	
model compounds	LCF results	(%)						
1. Fe in clays	0.462	40.42	0.421	35.77	0.438	37.60	0.366	28.48
2. Ferric oxides	0.532	46.54	0.603	51.23	0.583	50.04	0.713	55.49
7. Magnetite	0.064	5.60	0.079	6.71	0.103	8.84	0.161	12.53
9. Siderite	0.085	7.44	0.074	6.29	0.041	3.52	0.045	3.50
sum	1.258		1.177		1.165		1.285	
R-factor		0.0126	0.0106		0.0088		0.0087	
Chi-square		0.4647		0.2727		0.2376	0.2672	
Reduced chi-square		0.0027		0.0018		0.0016		0.0016



Figure SI- 22 XANES spectra of reference compound utilized for linear combination fit analysis of S K edge XANES



Figure SI- 23 XANES (left) and EXAFS (right) spectra of reference compounds used for linear combination fit analysis of Fe K edge



Figure SI- 24 Uranium L<sub>III</sub> edge XANES of RABS sediments after the bioreduction phase under sulfate-reducing conditions (SRC) (Col. No. 2 and 4) and iron-reducing conditions (IRC) (Col. No. 6)



Figure SI- 25 Fourier transforms of uranium L<sub>III</sub> edge EXAFS spectra (full lines) and shell-by-shell fits (black dotted lines) of SRC2, SRC4, and IRC6. Vertical black dotted lines represent the main peak from the paths fitted in the models.



Figure SI- 26 Uranium L<sub>III</sub> edge EXAFS spectra (full lines) and shell-by-shell fits (black dotted lines) of SRC2, SRC4, and IRC6.



Figure SI- 27 Iron K edge EXAFS of RABS before and after the bioreduction phase under sulfate-reducing conditions (SRC4) and iron-reducing conditions (IRC6).



Figure SI- 28 Net production of  $H_2O_2$  over time in SRC and IRC sediments during oxidation experiments in batch reactors in the presence and absence of SOD and CAT under oxic conditions (a) and suboxic conditions (b). Note the difference in y-axis range for oxic and suboxic experiments.



Figure SI- 29 Uranium speciation over time during the batch oxidation experiment under suboxic conditions for (a) SRC sediments and (b) IRC sediments. Solid lines represent the experiment with no amendment and dotted lines represent the amendment of SOD and CAT



Figure SI- 30 Cumulative uranium released over time in flow-through experiments in SRC and IRC sediments with low and high bicarbonate loading under (a) oxic conditions and (b) suboxic conditions.



Figure SI- 31 Dissolution of S in RAGW over time during SRC oxidation in flowing through CSTR reactors at 10 and 1 mM HCO3.



Figure SI- 32 Sulfur speciation over time in the flow-through experiment expressed as percentage based on the initial content of S by XRF, on S K edge LCF XANES speciation, and SO4<sup>2-</sup> measured in solution.



Figure SI- 33 U(VI) dissolution rate in oxic conditions in batch reactors under oxic conditions in SRC sediments (a) and IRC sediments (b). Blue dots represent data; yellow dots represent the control experiments with SOD and CAT in RAGW. Dashed lines represent the models based on the estimated constants of dissolution



Figure SI- 34 U(VI) dissolution rate in suboxic conditions in batch reactors under oxic conditions in SRC sediments (a) and IRC sediments (b). Blue dots represent data; yellow dots represent the control experiments with SOD and CAT in RAGW. Dashed lines represent the models based on the estimated constants of dissolution



Figure SI- 35 S K-edge XANES speciation in SRC sediments before and after oxidation in the batch experiment under oxic conditions. Full line represents the data. Dashed lines are the models.



Figure SI- 36 S K-edge XANES speciation in SRC sediments before and after oxidation in the batch experiment under oxic conditions in flowing through CSTR reactor at 10 mM HCO3. Full line represed the dataa. Dashed lines are the models.

$$R - factor = \frac{\sum (X(k)_{experimental \ data} - X(k)_{fit})^2}{\sum (X_{experimental \ data})^2}$$

Equation S 1 definition of R-factor in LCF analysis

$$X_{R}^{2} = \frac{N_{ind}}{[(N_{ind} - N_{p})n]} \sum_{i=1,n} [k^{3}x(k)_{i} - k^{3}x(k)calc_{i}]^{2}/[1 + \varepsilon(k)i]$$

Equation S 2 definition of reduced Chi squared for the assessment of the goodness of the first order kinetic dissolution reaction.

$$[U(VI)]_{dissolved} = [U(IV)]_{NCU4,0}(1 - e^{-kt})$$

Equation S 3 First order kinetic equation for dissolution of oxidized U(VI)

$$X^{2} = \sum_{i=1}^{n} \frac{([U(VI)]_{experimental \ data} - [U(VI)]_{model})^{2}}{[U(VI)]_{experimental \ data}}$$

Equation S 4 definition of Chi squared for the assessment of the goodness of the first order kinetic dissolution reaction.

# Annex 3 Supporting information Chpt. 4

Table SI- 25 Combinatorial results of linear combination fit analysis of end products of reduction and absorption experiment in Athena with three reference standards (Figure SI 1). The goodness of the fit is evaluated on the R-factor and X<sup>2</sup> as defined in Equation SI 1 and Equation SI 2. The best fits are In red.

				N(VI) [%]		N(V) [%]		N(IV)[%]		
R fac-	chi	n var	scale	weight	err	weight	err	weight	err	
tor	sqr		þλ							
0.00055	0.032	m	7			25		75	-	
0.00059	0.034	4	Ч	0		2 27	2	73	H	
0.00168	0.097	m	7	19		1		81	-	
0.01828	1.056	£	H	29		9 72	6			
0.00038	0.023	m	-			34		99	-	
0.00039	0.023	4	-	0		1 35		99	-	
0.00220	0.129	m	7	27		1		73	7	
0.01332	0.784	£	H	30		7 71	8			
0.00035	0.021	m	-			19		81		
0.00036	0.022	4	Ļ	0		1 21		80	-	
0.00103	0.061	m	7	15		1		85	-	
0.02135	1.267	£	Ļ	36		9 65	10			
0.00037	0.022	m	7			25		75		
0.00037	0.022	4	Ļ	0		1 26		74	-	
0.00132	0.078	m	7	21		1		79	7	
0.01739	1.034	£	-	35		99 66	6			
0.00067	0.019	m	-	66		0	2			
0.00067	0.019	4	-	66		2	2	0	-	
0.00113	0.031	Υ	1	100		0		0	0	
0.01054	0.289	ε	1			78	ъ	22	S	
0.00016	0.005	4	7	83		1		13		
0.00018	0.005	m	1	86		1		13	-	
0.00064	0.018	£	Ļ	91		2	2			
0.00590	0.166	m	-			69	4	31	4	

Annex 3 Supporting information Chpt. 4

Table SI- 26 Combinatorial results of linear combination fit analysis (LCF) of U Lin edge XANES with and without U(V) standard. The range of the fit was -20 to 40 eV relative to the first derivative E<sub>0</sub>. The goodness of the fit is evaluated on the R-factor and X<sup>2</sup> as defined in Equation SI 1 and Equation SI 2. The best fit include U(V) standards. The best fits are In red.

					) U	(1)	Ď	2	)n	<u>&gt;</u>
sample name	time [h]	Rf	chi	chi sqr	[%]	err	[%]	err	[%]	err
B1	0.5	2.39E-05	6.30E-06	8.50E-04	64	0.5	24	0.4	10	0.2
		5.37E-04	1.41E-04	1.92E-02	87	1.1			11	1.1
B3	œ	<b>3.89E-05</b>	1.03E-05	1.41E-03	60	0.6	26	0.6	13	0.3
		6.46E-04	1.69E-04	2.33E-02	85	1.2			14	1.2
B6	6	2.29E-05	6.10E-06	8.10E-04	43	0.5	39	0.4	17	0.2
		1.39E-03	3.69E-04	4.91E-02	80	1.7			19	1.7
B10	30	3.06E-05	8.20E-06	1.12E-03	42	0.6	43	0.5	15	0.3
		1.66E-03	4.38E-04	6.04E-02	82	1.9			16	1.9

Table SI- 27 Results of bulk U L<sub>III</sub> edge XANES linear combination fit of U(VI) reduction in batches containing Fe<sub>3</sub>O<sub>4</sub> or PO4 sorbed magnetite at various U concentration and U(VI) adsorption on  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>. (-) standard not required for the fit based on the X<sup>2</sup> (Table SI-1). Values in parenthesis are the uncertainties of the LCF from Athena<sup>12</sup>.

sample name	U:Fe [M:M]	U(VI) <sup>*</sup> [%]	U(V) * [%]	U(IV) * [%]	R factor
Fe₃O₄ (200 μM)	0.04	-	25(1)	75(1)	5.52E-04
Fe₃O₄ (80 μM)	0.016	-	34(1)	66(1)	3.84E-04
PO4 <sup>2-</sup> -Fe <sub>3</sub> O <sub>4</sub> (80 μM) repl.1	0.016	-	19(1)	81(1)	3.54E-04
PO4 <sup>2-</sup> -Fe <sub>3</sub> O <sub>4</sub> (80 μM) repl.1	0.016	-	25(1)	75(1)	3.69E-04
γ-Fe <sub>2</sub> O <sub>3</sub> (140 μM)	0.028	99(2)	0(2)	-	6.67E-04
γ-Fe <sub>2</sub> O <sub>3</sub> (80 μM)	0.016	83(1)	4(1)	13(1)	1.64E-03

\* Determined by LCF of bulk L<sub>III</sub> edge XANES spectra using uranyl adsorbed on ferrihydrite as standard for U(VI) and U(IV) reduced by  $Fe_3O_4$  for U(IV). Reference standards are presented in the supporting information. The range of the fit was -30 to 40 eV relative to the maximum first derivative (E<sub>0</sub>). The goodness of the fit is measured by R-factor as defined in the SI.

time [h]	dissolved U [µM]	C/C₀[%]	δ <sup>238</sup> U [‰]	U(VI)* [%]	U(V) * [%]	U(IV)* [%]	R factor
0.5	9.21	19.0%	0.11(0.06)	65(0.5)	24(0.5)	10(0.5)	2.64E-05
3	7.41	13.3%	0.12(0.08)	60(0.6)	26(0.6)	13(0.2)	4.19E-05
9	1.63	1.7%	0.3(0.03)	43(0.5)	39(0.4)	17(0.2)	2.37E-05
30	0.50	0.4%	0.29(0.07)	42(0.5)	43(0.5)	15(0.3)	3.18E-05

Table SI- 28 U(VI) reduction in separate batches containing 5mM Fe as Fe3O4 and 80  $\mu$ M U; concentration of dissolved uranium over time, Isotopic fractionation of U ( $\delta$ 238U) remaining in the aqueous phase in per mill ( $\infty$ ) and results of the linear combination fit (LCF) analysis by bulk U LIII-edge XANES in percentages (%). The values in parenthesis are the uncertainties of the LCF from Athena<sup>12</sup>.

\* Determined by LCF of bulk L<sub>III</sub> edge XANES spectra using uranyl adsorbed on ferrihydrite as standard for U(VI) and U(IV) reduced by  $Fe_3O_4$  for U(IV). Reference standards are presented in the supporting information. The range of the fit was -30 to 40 eV relative to the maximum first derivative (E<sub>0</sub>). The goodness of the fit is measured by R-factor as defined in the SI.

size [nm]	pr1	pr2
Row Labels	Count of Feret	Count of Feret
<0 or (blank)		
0-5	25	7
5-10	93	158
10-15	184	282
15-20	188	168
20-25	108	63
25-30	52	16
30-35	50	5
35-40	13	
40-45	10	1
45-50	4	
50-55	3	
55-60	3	
70-75	2	
Grand To- tal	735	700

### Table SI- 29 size distribution of magnetite nanoparticles in 2 batches



Figure SI- 37 Uranium  $L_{\mbox{\tiny III}}$  reference standards for XANES linear combination fit analysis



Figure SI- 38 Derivative of Uranium L<sub>III</sub> reference standards for XANES linear combination fit analysis



Figure SI- 39 U concentration (a) and U isotopic fractionation (b) in solution over time during U reduction by magnetite in separate batches


Figure SI – 40 Normalized bulk U LIII-edge XANES spectra of selected samples (black line), their LCFs (red dashed line) using U(VI) adsorbed on ferrihydrite as standard for U(VI) (blu line), biogenic UO<sub>2</sub> as standard for U(IV) (dark red). Fit results are reported in



Figure 4.4 Normalized bulk U LIII-edge XANES spectra of selected samples (black line), their LCFs (red dashed line) using U(VI) adsorbed on ferrihydrite as standard for U(VI) (blue), biogenic UO2 as standard for U(IV) (yellow) and U3O8 as standard for U(V) and U(VI) (dark red). Fit results are reported in Table 4.2.

Table 4.2.



Figure SI- 40 Particle size distribution in sample nr. 1, results of size measurements are reported in Table SI-3



Figure SI- 41 Particle size distribution in sample nr. 2, results of size measurements are reported in Table SI-3



Figure SI- 42 examples of diffraction pattern of magnetite from sample pr1 (a) and sample pr2 (b

$$R - factor = \frac{\sum (X(k)_{experimental \ data} - X(k)_{fit})^{2}}{\sum (X_{experimental \ data})^{2}}$$

Equation SI 1 definition of R-factor in LCF analysis

$$X_r^2 = \frac{N_{ind}}{vn} \sum_{i=1}^n \frac{(k^3 X(k)_{experimental \ data,i} - k^3 X(k)_{fit,i})^2}{\varepsilon_i^2}$$

Equation SI-2 Definition of reduced Chi-square  $Xr^2$ 

# Curriculum vitae

## **Contact information**

Luca Loreggian

Rue des Fosses 35

CH-1110 Morges

I.loreggian@gmail.com

+41788320204

# **Personal information**

CitizenshipItalian (Swiss residence permit: B)Family statussingleDate of birth24.01.1986

# **Employment history**

#### Education

2014-2012	Master of Science in Environmental Engineering University of Padue (Italy)
2012-2009	Bachelor of Science in Environmental Engineering University of Padue (Italy)
2009-2006	Bachelor of Science in Forestry Science and Technology University of Padue (Italy)

## **Professional qualifications**

- Synchrotron based method: X-ray absorption spectroscopy
- Inductively coupled mass spectroscopy
- Inductively coupled plasma optical emission
- Ion chromatography
- Methodological ability to conduct research projects
- Oral, writing and presenting skills
- Scientific curiosity and enthusiasm toward solving environmental challenges
- Italian (Mother tongue), English (Professional), French (Advance)

#### Award

October 2018 Best contribution Ex-Aequo Uranium Biogeo International conference Uranium biogeochemistry

#### **Publications**

Polesel, F.; Torresi, E.; Loreggian, L.; Escola Casas, M.; Christensson, K. B.; Plosz, B. Removal of pharmaceuti-

cals in pre-denitrifying MBBR – Influence of organic substrate availability in single- and three-stage configu-

rations. Water Research 2017 Oct 15; 123:408-419

Carla Perez-Mon, Aislinn A. Boylan, Laurent Guillard, Niels Burzan, Luca Loreggian, Markus Maisch, Andreas

Kappler, James M. Byrne and Rizlan Bernier-Latmani. H2 fuelled microbial metabolism in Opalinus Clay. Sub-

mitted manuscript

Maria P. Asta, Yuheng Wang, Pierre Le Pape, Manon Frutschi, Karen Viacava, **Luca Loreggian**, Phu Le Vo, Ana M Fernández, Guillaume Morin and Rizlan Bernier-Latmani. Microbially mediated release of As from Mekong Delta peat sediment. Environmental Science and Technology. *Submitted manuscript*.

Ce document a été imprimé au Centre d'impression EPFL, imprimerie climatiquement neutre, certifiée myClimate.