

UO₂ REOXIDATION IN THE PRESENCE OF CHELATORS AND Fe(III)
(HYDR)OXIDES

by

Crystal Lee Girardot

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Chemical Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 2010

©COPYRIGHT

by

Crystal Lee Girardot

2010

All Rights Reserved

APPROVAL

of a thesis submitted by

Crystal Lee Girardot

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency and is ready for submission to the Division of Graduate Education.

Dr. Brent Peyton

Approved for the Department of Chemical and Biological Engineering

Dr. Ron Larsen

Approved for the Division of Graduate Education

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Crystal Lee Girardot

November 2010

ACKNOWLEDGMENTS

I would first like to acknowledge my primary advisor, Dr. Brent Peyton, for taking a chance on financially supporting my education when I had no prior research experience. I am deeply thankful and hope I exceeded any expectations. I would also like to thank Dr. Brandy Stewart for mentoring me throughout the final year of my master's education. She provided answers to my many questions, support, as well as friendship. I was very fortunate to have her guidance along the way especially when Dr. Peyton was unavailable. She helped me greatly with the writing of this thesis.

I would like to thank Dr. Abigail Richards for all her advice and guidance throughout my first year as a graduate research assistant. She helped me get on the right track and was encouraging when experiments were not going the way as planned. I would also like to thank Dr. Robin Gerlach for his advice and all his help with the ICP-MS and running all my many samples. I would also like to thank all the faculty and students in the Chemical and Biological Engineering department, the Center for Biofilm Engineering, as well as the Peyton research group for all of their help and support I have received. Special thanks to Ari Staven for her editing of this thesis as well as Karen Moll for helping me on the microscope.

Lastly, I would like to thank my family and friends for their love and support. Lastly, thank you Porter, my dog, for being so good while I was busy writing my thesis and not having time to play with you and Arlo, my cat, for cuddling with me on my lap while typing away.

TABLE OF CONTENTS

1. INTRODUCTION	1
Uranium	1
Chemical Properties.....	2
Uranium Removal Techniques and Methods	3
Uranium Reduction.....	4
Uranium Reoxidation.....	6
Iron.....	9
Fe(III) (hydr)oxides	10
Role in Uranium Redox Reactions	11
Chelators	13
Citrate	15
EDTA and NTA.....	16
Siderophores	19
DFB.....	19
Summary.....	22
2. METHODS	23
Experimental Goals	23
Materials and Methods	23
Materials	23
Synthesis of Iron (Hydr)oxide-Coated Sands	25
Analytical Methods.....	27
Aqueous U(VI)	27
Total Dissolved U	28
Total Dissolved Iron	29
Dissolution Experiments of Fe(III) (Hydr)oxides in the Presence of Chelators ...	29
Dissolution Experiments of Biogenic UO ₂ in the Presence of Chelators	30
UO ₂ Reoxidation Experiments	31
Fe(III) (Hydr)oxides	31
Ferric Chloride.....	31
Fe(III) Bound to Chelators.....	32
Rate Constant Calculations.....	32
3. RESULTS AND DISCUSSIONS.....	34
Dissolution of UO ₂ and Fe(III) (hydr)oxides.....	34
Dissolution Experiments with DFB.....	34
Dissolution Experiments with Citrate.....	39
Dissolution Experiments with EDTA and NTA	44

TABLE OF CONTENTS – CONTINUED

UO ₂ Reoxidation Experiments	50
Reoxidation of UO ₂ by Ferric Chloride.....	50
Reoxidation of UO ₂ by DFB Bound to Fe(III)	51
Reoxidation of UO ₂ by Ferric Citrate.....	53
Reoxidation of UO ₂ in the Presence of DFB and Fe(III) (Hydr)oxides	54
UO ₂ Reoxidation in the Presence of DFB and Ferrihydrite	54
UO ₂ Reoxidation in the Presence of DFB and Goethite or Hematite.....	58
Reoxidation of UO ₂ in the Presence of Citrate and Fe(III) (Hydr)oxides	61
UO ₂ Reoxidation in the Presence of Citrate and Ferrihydrite	61
UO ₂ Reoxidation in the Presence of Citrate and Goethite or Hematite.....	63
Reoxidation of UO ₂ in the Presence of EDTA/NTA and Fe(III) (Hydr)oxides	66
UO ₂ Reoxidation in the Presence of EDTA and Fe(III) (Hydr)oxides.....	66
UO ₂ Reoxidation in the Presence of NTA and Fe(III) (Hydr)oxides	68
 4. CONCLUSIONS AND FUTURE WORK.....	 70
Conclusions.....	70
Future Work.....	72
 REFERENCES	 75
 APPENDICES	 82
APPENDIX A: Raw Data for UO ₂ Dissolution Experiments.....	83
APPENDIX B: Raw Data for UO ₂ Reoxidation Experiments	99
APPENDIX C: Raw Data for Fe(III) (Hydr)oxide Dissolution Experiments	139
APPENDIX D: Investigating Siderophore Production by Sulfate-Reducing Bacteria	167

LIST OF TABLES

Table	Page
1.1. Redox reactions for UO_2 with Fe(III) (hydr)oxides	12
1.2. Stability constants for DFB, citrate, EDTA, and NTA.....	21
3.1. Rate constant results for Fe(III) (hydr)oxide dissolution experiments.....	36
3.2. Rate constant results for UO_2 dissolution experiments.....	38
3.3. Rate constant results for UO_2 reoxidation experiments	57
4.1. List of possible chelators to test for UO_2 reoxidation.....	73
A1. Raw data for UO_2 dissolution with DFB at 0mM bicarbonate.....	86
A2. Raw data for UO_2 dissolution with DFB at 3mM bicarbonate.....	87
A3. Raw data for UO_2 dissolution with DFB at 10mM bicarbonate.....	89
A4. Raw data for UO_2 dissolution with citrate at 0mM bicarbonate.....	91
A5. Raw data for UO_2 dissolution with citrate at 3mM bicarbonate.....	93
A6. Raw data for UO_2 dissolution with citrate at 10mM bicarbonate.....	95
A7. Raw data for UO_2 dissolution with EDTA at 10mM bicarbonate	97
A8. Raw data for UO_2 dissolution with NTA at 10mM bicarbonate	98
B1. Raw data for UO_2 reoxidation with DFB and goethite or hematite at 0mM bicarbonate.....	100
B2. Raw data for UO_2 reoxidation with DFB and goethite at 3mM or 10mM bicarbonate.....	103
B3. Raw data for UO_2 reoxidation with DFB and hematite at 3mM or 10mM bicarbonate.....	106
B4. Raw data for UO_2 reoxidation with DFB and ferrihydrite at 3mM or 10mM bicarbonate.....	109

LIST OF TABLES – CONTINUED

Table	Page
B5. Raw data for UO_2 reoxidation with ferric chloride	113
B6. Raw data for UO_2 reoxidation with DFB bound to Fe(III) (0.625mM).....	115
B7. Raw data for UO_2 reoxidation with DFB bound to Fe(III) (1.25mM)	117
B8. Raw data for UO_2 reoxidation with ferric citrate.....	118
B9. Raw data for UO_2 reoxidation with citrate and ferrihydrite at 0mM, 3mM, or 10mM bicarbonate	120
B10. Raw data for UO_2 reoxidation with citrate and hematite at 0mM, 3mM, or 10mM bicarbonate	124
B11. Raw data for UO_2 reoxidation with citrate and goethite at 0mM, 3mM, or 10mM bicarbonate	127
B12. Raw data for UO_2 reoxidation with EDTA and ferrihydrite, goethite, or hematite at 0mM, 3mM, or 10mM bicarbonate.....	130
B13. Raw data for UO_2 reoxidation with various chelators and ferrihydrite at 10mM bicarbonate	133
B14. Raw data for UO_2 reoxidation with NTA and ferrihydrite, goethite, or hematite at 0mM, 3mM, or 10mM bicarbonate.....	136
C1. Raw ICP-MS data for UO_2 dissolution by DFB with 0mM bicarbonate at day 20.....	140
C2. Raw ICP-MS data for UO_2 dissolution by DFB with 3mM or 10mM bicarbonate at day 6	141
C3. Raw ICP-MS data for UO_2 dissolution by DFB with 3mM or 10mM bicarbonate at day 21	142
C4. Raw ICP-MS data for UO_2 dissolution by DFB with 3mM or 10mM bicarbonate at day 35	143

LIST OF TABLES – CONTINUED

Table	Page
C5. Raw ICP-MS data for dissolution of ferrihydrite, goethite, and hematite by DFB.....	145
C6. Raw ICP-MS data for dissolution of ferrihydrite, goethite, and hematite by citrate	148
C7. Raw ICP-MS data for dissolution of ferrihydrite, goethite, and hematite by EDTA with 10mM bicarbonate	151
C8. Raw ICP-MS data for dissolution of ferrihydrite, goethite, and hematite by NTA with 10mM bicarbonate.....	155
C9. Raw ICP-MS data for dissolution of ferrihydrite, goethite, and hematite by EDTA with 0mM bicarbonate	159
C10. Raw ICP-MS data for dissolution of ferrihydrite, goethite, and hematite by NTA with 0mM bicarbonate.....	163
D1. Raw data for Coomassie, CAS, Csàky, and Arnow assays in <i>D. vulgaris</i> cultures with low iron	205
D2. Raw data for Coomassie, CAS, Csàky, and Arnow assays in <i>D. vulgaris</i> cultures with no iron/AW	206
D3. Raw data for Coomassie, CAS, Csàky, and Arnow assays in <i>D. vulgaris</i> cultures with 6.25nM and 0.625nM iron	207
D4. Raw data for Coomassie, CAS, Csàky, and Arnow assays in <i>D. vulgaris</i> cultures with no iron	208
D5. Raw data for Coomassie, CAS, Csàky, and Arnow assays in <i>D. vulgaris</i> cultures with pyruvate/sulfate medium.....	209
D6. Raw data for siderophore detection on <i>D. vulgaris</i>	210
D7. Raw data for siderophore detection on <i>D. vulgaris</i> after 2.5% zinc treatment.....	213
D8. Raw data for siderophore detection on <i>D. vulgaris</i> cultures (7 days) after treatment with 5.2% zinc	213

LIST OF TABLES – CONTINUED

Table	Page
D9. Raw data for siderophore detection on <i>D. vulgaris</i> cultures (15 days) after treatment with 5.2% zinc	214
D10. Raw data for siderophore detection on <i>D. vulgaris</i> cultures (15 days), no Fe after treatment with 5.2% zinc	214
D11. Raw data for CAS assay on <i>D. vulgaris</i> after treatment with H ₂ O ₂	214
D12. Raw data for CAS assay on controls treated with 2.6% zinc	215
D13. Raw data for CAS assay on controls treated with 2.6% silver	215
D14. Raw data for CAS assay on controls treated with 5.2% zinc	216
D15. Raw data for CAS assay on controls purged with air	217
D16. Raw data for CAS assay on controls in no iron LS4D purged with air	218
D17. Raw data for CAS assay on controls in no iron LS4D purged with air	219
D18. Raw data for CAS assay on <i>D. vulgaris</i> (7 days) purged with air	220
D19. Raw data for CAS assay on <i>D. vulgaris</i> (15 days) purged with air	220
D20. Raw data for CAS assay on <i>D. vulgaris</i> eluent (7 and 12 days) purged with air ...	221
D21. Raw data for CAS assay on G20 treated with H ₂ O ₂	223
D22. Raw data for CAS assay on G20 treated with 2.6% zinc	223
D23. Raw data for CAS assay on G20 purged with air	224

LIST OF FIGURES

Figure	Page
1.1. Eh-pH diagram for U	11
1.2. The structure of citric acid	15
1.3. The structure of citrate bound to Fe(III)	15
1.4. The structure of citrate bound to U(VI)	15
1.5. The structure of EDTA and NTA	17
1.6. The structure of EDTA bound to U(VI)	17
1.7. The structure of EDTA bound to Fe(III)	17
1.8. The structure of NTA bound to U(VI)	18
1.9. The structure of NTA bound to Fe(III)	18
1.10. The structure of DFB	20
1.11. The structure of DFB bound to Fe(III)	20
1.12. The structure of DFB bound to U(VI)	21
3.1. Dissolution of ferrihydrite, goethite, and hematite by DFB	35
3.2. Dissolution of UO ₂ by DFB at 3mM or 10mM bicarbonate	37
3.3. Dissolution of ferrihydrite, goethite, and hematite by citrate	40
3.4. Dissolution of UO ₂ by citrate at 0mM, 3mM, or 10mM bicarbonate	42
3.5. Dissolution of UO ₂ by citrate at 0mM, 3mM, or 10mM bicarbonate	43
3.6. Dissolution of ferrihydrite, goethite, and hematite by EDTA with 0mM bicarbonate	45
3.7. Dissolution of ferrihydrite, goethite, and hematite by EDTA with 10mM bicarbonate	46

LIST OF FIGURES – CONTINUED

Figure	Page
3.8. Dissolution of ferrihydrite, goethite, and hematite by NTA with 0mM bicarbonate.....	47
3.9. Dissolution of ferrihydrite, goethite, and hematite by NTA with 10mM bicarbonate.....	48
3.10. Dissolution of UO_2 by EDTA and NTA with 10mM bicarbonate	49
3.11. UO_2 reoxidation by aqueous Fe(III) versus pH	51
3.12. UO_2 reoxidation by Fe(III) bound to DFB versus pH	52
3.13. UO_2 reoxidation by ferric citrate versus pH	53
3.14. UO_2 reoxidation in the presence of DFB and ferrihydrite at 0mM, 3mM, or 10mM bicarbonate	55
3.15. UO_2 reoxidation in the presence of DFB and goethite at 0mM, 3mM, or 10mM bicarbonate	59
3.16. UO_2 reoxidation in the presence of DFB and hematite at 0mM, 3mM, or 10mM bicarbonate	60
3.17. UO_2 reoxidation in the presence of citrate and ferrihydrite at 0mM, 3mM or 10mM bicarbonate.....	62
3.18. UO_2 reoxidation in the presence of citrate and goethite at 0mM, 3mM or 10mM bicarbonate.....	64
3.19. UO_2 reoxidation in the presence of citrate and hematite at 0mM, 3mM or 10mM bicarbonate.....	65
3.20. UO_2 reoxidation in the presence of EDTA and ferrihydrite, goethite, or hematite with 10mM bicarbonate.....	67
3.21. UO_2 reoxidation in the presence of NTA and ferrihydrite, goethite, or hematite with 10mM bicarbonate.....	69
A1. KPA-11 calibration curve for U(VI).....	84

LIST OF FIGURES – CONTINUED

Figure	Page
A2. KPA-11 calibration curve for total dissolved U	85
C1. U calibration curve from ICP-MS data.....	140
C2. Dissolution of ferrihydrite, goethite, and hematite by DFB	144
C3. Iron calibration curve from ICP-MS data.....	144
D1. Growth curve of <i>D. vulgaris</i> in various iron concentrations	197
D2. Purging controls with compressed air.....	199
D3. CAS assay on G20 cultures (7 and 15 days).....	200
D4. Siderophore production from G20.....	200
D5. CAS assay on <i>D. vulgaris</i> cultures (7 and 15 days).....	201
D6. Siderophore production from <i>D. vulgaris</i>	201
D7. Growth curve of <i>D. vulgaris</i> with 62.5 μ M and 6.25 μ M Fe	210
D8. Growth curve of <i>D. vulgaris</i> with 0.625 μ M and 0.0625 μ M Fe	211
D9. Growth curve of <i>D. vulgaris</i> with no Fe	211
D10. Growth curve of <i>D. vulgaris</i> in pyruvate/sulfate medium	212
D11. Growth curve tracking protein of <i>D. vulgaris</i> in pyruvate/sulfate medium.....	212
D12. Siderophore production by <i>D. vulgaris</i> purged with air	222
D13. Siderophore production by <i>D. vulgaris</i> (7 and 15 days) treated with 5.2% zinc	222

ABSTRACT

A proposed method of limiting uranium (U) migration is the reduction of soluble U(VI) to U(IV) with subsequent precipitation of uraninite, $\text{UO}_2(\text{s})$. However, microbially reduced UO_2 may be susceptible to reoxidation by environmental factors, with Fe(III) (hydr)oxides playing a significant role. Little is known about the role that organic compounds such as Fe(III) chelators play in the stability of reduced U. Here we investigate the impact of DFB (desferrioxamine B), citrate, EDTA (ethylenediaminetetraacetic acid), and NTA (nitrilotriacetic acid) on biogenic UO_2 reoxidation with ferrihydrite, goethite, or hematite. Experiments were run in anaerobic batch systems in PIPES buffer (pH 7) with bicarbonate for approximately 80 days. U(VI) concentrations were measured using a kinetic phosphorescence analyzer. Results showed EDTA accelerated UO_2 reoxidation the most at an initial rate of $9.5\mu\text{M day}^{-1}$ according to the non-zero-order rate equation $R = k_I [\text{chelator}]$, with a rate constant k_I of 0.049, 0.044, and 0.046 day^{-1} for ferrihydrite, goethite, and hematite, respectively. NTA accelerated UO_2 reoxidation with ferrihydrite at a rate of $4.8\mu\text{M day}^{-1}$ with $k_I = 0.026\text{ day}^{-1}$; rates were less with goethite and hematite (0.66 and $0.71\mu\text{M day}^{-1}$ with $k_I = 0.0038$ and 0.0004 day^{-1} , respectively). Citrate increased UO_2 reoxidation with ferrihydrite at a rate of $1.8\mu\text{M day}^{-1}$ with $k_I = 0.009\text{ day}^{-1}$, but did not increase the extent of reaction, and no reoxidation occurred with goethite or hematite. DFB inhibited UO_2 reoxidation with ferrihydrite, and no reoxidation occurred with goethite or hematite. In all cases, bicarbonate increased the rate and extent of UO_2 reoxidation with ferrihydrite in the presence and absence of chelators. The highest rate of UO_2 reoxidation occurred when the chelator promoted UO_2 and Fe(III) (hydr)oxide dissolution as demonstrated with EDTA. When UO_2 dissolution did not occur, UO_2 reoxidation likely proceeded through an aqueous Fe(III) intermediate. The rate of UO_2 reoxidation was dependent on the stability constant between chelator and Fe(III), with DFB likely inhibiting reoxidation due to a very high stability constant ($\log K = 30.6$). These results indicate that common chelators found in U contaminated sites can play a significant role in mobilizing U affecting efforts for bioremediation.

CHAPTER 1

INTRODUCTION

The goal of this project is to gain insight into abiotic reactions involving Fe(III) that might cause bio-reduced uraninite (UO_2) to undergo reoxidation, become mobile and migrate into groundwater causing environmental contamination. Previous studies have shown total UO_2 reoxidation will occur when free excess aqueous Fe(III) is present; however, when Fe(III) is present as an Fe(III) (hydr)oxide, then only partial UO_2 oxidation occurs (Sani et al., 2005; Ginder-Vogel et al., 2006). For this project, different chelators were tested for their influence on biogenic UO_2 and Fe(III) (hydr)oxide dissolution at circumneutral pH. They were also investigated for their influence on UO_2 reoxidation in the presence of goethite, hematite, or ferrihydrite. The rates of UO_2 reoxidation were measured and compared between the chelators desferrioxamine B (DFB), citrate, ethylenediaminetetraacetic acid (EDTA), and nitrilotriacetic acid (NTA).

Uranium

Uranium (U) is the forty-ninth most abundant element in the earth's crust and is found in most rocks, soil, and water. Unfortunately, due to many anthropogenic activities (U mining and milling, fuel production, and nuclear research), the amount of U localized to specific areas in the United States has resulted in extensive environmental contamination (Wall and Krumholz, 2006). One hundred and twenty nuclear weapon production sites covering 7,280 km^2 were shut down at the end of the cold war. A plan to remediate the impacted environments was necessary (Wall and Krumholz, 2006). Of

these sites, U is the most common radionuclide (Lloyd and Lovley, 2001) making this contaminant a primary target for clean up by the Department of Energy (DOE) as well as increasing interest in understanding U's subsurface fate.

Chemical Properties

Natural and depleted U are composed of three isotopes: ^{238}U , ^{235}U , and ^{234}U , are chemically identical, and considered more toxic chemically than from radioactivity.

Depleted U is roughly 40% less radioactive than natural U (Craft et al., 2004). The main radiation hazard occurs from ingesting or inhaling U, as alpha emitters like U have little penetrating abilities.

The mobility of U is mainly controlled by complexation and reduction/oxidation (redox) reactions in the environment. U has five different oxidation states ranging from +2 (completely reduced) to +6 (completely oxidized), although only the +4 and +6 states are stable in natural settings (Craft et al., 2004). In general, hexavalent U [U(VI)] compounds are considered the most soluble and the oxidation of U(IV) to U(VI) is regarded as mobilizing U. Tetravalent U [U(IV)] compounds are considered insoluble and the reduction of U(VI) to U(IV) is considered immobilizing U due to the formation and precipitation of U(IV) minerals such as UO_2 (Langmuir, 1978). Recently, the reduction of U(VI) to U(IV) has led to the discovery of molecular U(IV), which may also be susceptible to reoxidation, like UO_2 , since oxygen was shown to oxidize molecular U(IV) (Fletcher et al., 2010). The solubility and toxicity decrease with the reduction of U from an oxidation state of +6 to +4 (Gadd, 2000). It is important to understand what

factors determine U solubility as well as those that may contribute to its long-term stability to ensure minimal toxicity.

In oxygen-rich environments, U(VI) dominates and is primarily present as the uranyl ion, (UO_2^{2+}), which can form stable complexes with phosphate, calcium, and carbonate. Only in sufficiently reduced environments does U(IV) remain stable (Gavrilescu et al., 2009). Two common uranyl carbonates found in the environment are $\text{UO}_2(\text{CO}_3)_3^{4-}$ and $\text{UO}_2(\text{CO}_3)_2^{2-}$ which have high stability constants (log K values are 21.84 and 16.61 for $\text{UO}_2(\text{CO}_3)_3^{4-}$ and $\text{UO}_2(\text{CO}_3)_2^{2-}$, respectively) (Duquene et al., 2008). In calcium-rich environments, such as U contaminated sites where limestone (CaCO_3) is present, virtually all U found was complexed to calcium as either $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ or $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ (Brooks et al., 2003). U complexation to inorganic and organic compounds as well as redox reactions seems to control U mobility and fate in natural systems. Many of these complexation and redox reactions are influenced by microbial interactions such as the ability of bacteria to reduce U(VI) to UO_2 and bicarbonate concentrations increasing from microbial respiration that bind to U(VI) favoring mobilization (Lovley et al., 1991; Wan et al., 2005).

Uranium Removal Techniques and Methods

Many techniques have been proposed to clean up U from contaminated sites. Some examples include: immobilization, mobilization, biosorption and separation. U can be removed from solution biologically by sorption, through precipitation as a U(VI) compound, or by reductive precipitation as a U(IV) compound (Gorby and Lovley, 1992; Lovley and Coates, 1997; Gadd, 2000; Lloyd and Lovley, 2001; Sani et al., 2005). With

the help of U(VI) reduction to U(IV) by microorganisms leading to precipitation as UO_2 , U remediation by reductive precipitation may be cost effective in the deep subsurface compared to other techniques of U immobilization. It is thus important to identify the factors that control the stability of U to restrict its environmental impact.

Uranium Reduction

U reduction from U(VI) to U(IV) requires two electrons, and many compounds are capable of abiotically reducing U(VI) to U(IV) in the absence of bicarbonate including aqueous H_2S (at low pH), surface bound Fe(II), and sulfide minerals (Lovely et al., 1991; Wersin et al., 1994; Liger et al., 1999; Beyenal et al., 2004; Hua et al., 2006). The reduction of U(VI) to U(IV) results in immobilization through the precipitation of UO_2 . However, when U(VI) is bound to carbonate or calcium then these chemical reductants are less effective in reducing U(VI). Since uranyl carbonate $[\text{U(VI)}\text{-CO}_3]$ and uranyl calcium carbonate $[\text{U(VI)}\text{-Ca-CO}_3]$ complexes are the most dominant aqueous U species in the surface and subsurface (Brooks et al., 2003; Suzuki et al., 2003) abiotic U reduction by inorganic reductants can be unsuccessful in natural settings and reduction might only occur with the aid of microorganisms.

Many variables contribute to the rate of U(VI) reduction including Ca^{2+} ions; U forms stable complexes with calcium which can dramatically slow reduction of U(VI) by microorganisms (Brooks et al., 2003). Copper also displays inhibitory effects on microbial U reduction (Ganesh et al., 1999). U(VI) can form stable complexes with organic ligands, which also affects reduction rates. The rate of U(VI) reduction was slower when U(VI) was bound to a multidentate ligand such as citrate and malonate

compared to a monodentate ligand such as acetate (Ganesh et al., 1997; Ganesh et al., 1999). Thus, not only do certain compounds affect the rate of U(VI) reduction, but also the structure of the ligands present play an important role in U(VI) reduction.

Lovley et al. (1991) first showed microbial reduction of U(VI) with Fe(III)-reducing microorganisms (strain GS-15 and *Alteromonas putrefaciens*) and growth was supported by obtaining energy from electron transport to U(VI). Prior to Lovley et al. (1991), U(VI) reduction had been considered an abiotic reaction and microorganisms were considered to have an indirect role in reduction of U(VI) through bioproduction of sulfide and hydrogen which non-enzymatically reduced U(VI) (Langmuir, 1978). However, it has been shown that microorganisms enzymatically catalyze the reduction of U(VI) compared to the abiotic reduction of U(VI) in the presence of bicarbonate (Lovley et al., 1992). Also, the rate of U(VI)-CO₃ reduction by bioproduced sulfide is significantly reduced in the presence of Fe(III) (hydr)oxides due to the formation of iron-sulfide minerals (Abdelouas et al., 1998; Neal et al., 2001). Thus, in environmental settings, U(VI) reduction is likely enzymatic due to microorganisms.

Over thirty different types of bacteria have been recognized in their ability to enzymatically reduce U(VI) to U(IV) (Wall and Krumholz, 2006). One group of bacteria capable of U(VI) reduction is sulfate-reducing bacteria (SRB). Only the genus *Desulfovibrio* (*D*) of SRB has been shown to enzymatically reduce U(VI). *Desulfovibrio* include but are not limited to: *D. desulfuricans*, *D. vulgaris*, and *D. baculatus* (Tebo and Obraztsova, 1998; Suzuki et al., 2004). However, growth of *Desulfovibrio* was not supported using U(VI) as an electron acceptor (Lovley and Phillips, 1992; Lovley et al.,

1993b). Besides SRB, many types of bacteria are capable of reducing U(VI), and the list of bacteria capable of U(VI) reduction continues to grow.

Uranium Reoxidation

To ensure effective immobilization, U needs to be stable and resist being reoxidized to its soluble form (Wall and Krumholz, 2006). A number of studies have shown the ability of biogenic UO_2 to undergo reoxidation due to a number of different factors (Frazier et al., 2005; Gu et al., 2005; Sani et al., 2005; Wan et al., 2005; Ginder-Vogel et al., 2006; Ginder-Vogel et al., 2010).

Under aerobic conditions, U(VI) is the most stable form of U. Oxidants like oxygen and Mn oxides, which are able to gain two electrons from the oxidation reaction of UO_2 to U(VI), promote U remobilization (Fredrickson et al., 2002; Ulrich et al., 2009). Fe(III) is an oxidant capable of rapidly reoxidizing UO_2 in abiotic conditions (Sani et al., 2005). Organic soil compounds, or humic substances, accelerated UO_2 reoxidation in the presence of oxygen and have been shown to complex with U(IV) (Gu et al., 2005).

Other oxidants that can contribute to reoxidation of bio-reduced UO_2 include nitrate which is found in many U contaminated sites and can rapidly reoxidize UO_2 through the production of reactive intermediates (i.e., NO_2^- , NO, and N_2O) (Finneran et al., 2002). However, UO_2 oxidation by these nitrate intermediates can be inhibited if compounds such as acetate, aqueous Fe(II), H_2S , and iron-sulfide minerals are in excess compared to nitrate (Abdelouas et al., 2000; Senko, Suflita and Krumholz, 2005).

DFB, a siderophore, has been shown to increase dissolution of chemically synthesized UO_2 by forming a stable U(IV)-DFB complex that could lead to enhancing U

mobility (Frazier et al., 2005). 17-month-long column studies have demonstrated UO_2 reoxidation in the presence of active U(VI)-reducing microorganisms. Microbial respiration increases the bicarbonate concentration, which forms stable complexes with U (Wan et al., 2005). Oxidants, humic substances, bicarbonate, and siderophores are all potential compounds that can lead to biogenic UO_2 reoxidation to U(VI) as they are able to accept electrons from UO_2 , act as an electron shuttle, or form stable complexes with either U(VI) or U(IV) or both (Frazier et al., 2005; Gu et al., 2005; Wan et al., 2005).

Past studies appear to show Fe(III) (hydr)oxides play a role in biogenic UO_2 reoxidation (Nevin and Lovely, 2000; Sani et al., 2004; Sani et al., 2005; Senko et al., 2005a; Senko et al., 2005b; Wan et al., 2005). Senko et al. (2005a) observed oxidation of biogenic UO_2 with the release of Fe(II) in the presence of Fe(III) (hydr)oxide minerals, which were produced by nitrate-dependent Fe(II) oxidation. Biogenic UO_2 reoxidation in the presence of Fe(III) (hydr)oxides has also been shown to proceed under sulfate-reducing conditions as well as methanogenic conditions (Sani et al., 2004; Sani et al., 2005; Wan et al., 2005). Thermodynamically, the oxidation of biogenic UO_2 in the presence of Fe(III) (hydr)oxides is favorable under limited geochemical conditions (Ginder-Vogel et al., 2006). Ginder-Vogel et al. (2006) demonstrated that UO_2 oxidation is dependent on Fe(III) (hydr)oxide mineralogy, where goethite and hematite have a limited capacity to oxidize UO_2 versus ferrihydrite, which can lead to UO_2 oxidation. They also demonstrated that UO_2 oxidation increased with increasing calcium and bicarbonate concentration, but decreased with increasing aqueous Fe(II) and U(VI) concentrations (Ginder-Vogel et al., 2006).

Sani et al. (2005) showed that under abiotic conditions, aqueous Fe(III) oxidized UO_2 completely in less than 24 hours, while the Fe(III) (hydr)oxide, hematite, did not. However, in the presence of *Desulfovibrio desulfuricans* G20 (G20), the addition of Fe(III) (hydr)oxides allowed for biogenic UO_2 to be reoxidized once cultures were lactate limited. Hematite's reoxidation rates were greater than goethite or ferrihydrite. Currently, the exact mechanism is still unclear how sulfate-reducing activity affects U reoxidation in the presence of Fe(III) (hydr)oxides.

UO_2 reoxidation has been demonstrated in the presence of ferrihydrite with 3mM bicarbonate at circumneutral pH (Ginder-Vogel et al., 2006). No reoxidation occurred with goethite or hematite. Increasing bicarbonate concentrations lead to an increase of U(VI) concentration in the presence and absence of ferrihydrite (Ginder-Vogel et al., 2006). One mechanism proposed by Ginder-Vogel et al. (2010) in the reoxidation of biogenic UO_2 by ferrihydrite, appears to proceed through a soluble U(IV) intermediate, as the rate-controlling step, resulting in the production of Fe(II) and U(VI). Dissolution of UO_2 would result in soluble U(IV) which could then adsorb onto the surface of ferrihydrite and allow for electron transfer. Ginder-Vogel et al. (2010) also observed higher rates of UO_2 oxidation when conditions were tested that increased the solubility of UO_2 ($\text{pH} < 7$ with the minimum reoxidation rate occurring at $\text{pH} 7.2$) and decreased surface passivation ($\text{pH} > 7$, bicarbonate concentrations $> 500\mu\text{M}$). Factors promoting biogenic UO_2 dissolution may control UO_2 oxidation by Fe(III) (hydr)oxides. UO_2 oxidation by naturally occurring minerals and organic compounds could affect the rate of reoxidation, which is dependent on the environmental conditions. It is important to

identify which compounds and minerals can reoxidize UO_2 and possible mechanisms to ensure long term stability of biologically reduced UO_2 for bioremediation processes.

Iron

Iron is the fourth most abundant element in the earth's crust and is second only to aluminum among the metals. Iron can adopt either of two redox states, Fe(II) or Fe(III), which is influenced by its environment and both states form six-coordinate octahedral structures with either O, N, or S (Neilands, 1991). Although iron is abundant on Earth, it is considered biologically unavailable since iron exists mostly as highly insoluble Fe(III) (hydr)oxides in oxygen-rich environments at nearly neutral pH. Iron is essential for all microorganisms with the exception of *Lactobacilli* (Archibald, 1983), and microorganisms have developed sophisticated strategies to obtain this vital nutrient that is considered biologically unavailable.

Under anaerobic conditions, Fe(II) dominates and can be taken up readily by microorganisms. Under aerobic conditions, solutions of up to 100mM Fe(II) can be readily absorbed by microorganisms due to it being quite soluble (Neilands, 1991); however, Fe(II) is quickly oxidized to Fe(III) and forms insoluble Fe(III) (hydr)oxides. The solubility constant of these Fe(III) (hydr)oxides are very small (for ferrihydrite $\log K = -38$) in oxygen-rich environments at biological pH limiting free Fe(III) concentrations to 10^{-17} to 10^{-18} M (Neilands, 1993), and microorganisms need between 10^{-8} to 10^{-6} M iron for optimum growth, making iron virtually unavailable (Guerinot, 1994). Iron (II) can also be extremely toxic in aerobic conditions resulting in harmful Fenton-type reactions producing hydroxyl radicals capable of harming microorganisms (Touati, 2000;

Wandersman and Delepelaire, 2004), which leaves aerobic microbes in an environment where a vital nutrient is biologically unavailable or otherwise toxic. Consequently, microorganisms have developed sophisticated methods for meeting iron requirements. Some examples include: reducing Fe(III) to Fe(II), sequestering iron by the production and excretion of siderophores, developing methods for transporting siderophore-iron complexes into the cell, usage of iron storage proteins such as heme and transferrin, and/or abstaining from using iron altogether (Guerinot, 1994; Richards, 2007).

Fe(III) (hydr)oxides

In most environments, iron deficiency is the result of its low solubility at circumneutral pH and the low dissolution kinetics of Fe(III) (hydr)oxides. Iron is most often found as Fe(III) (hydr)oxides in terrestrial and aquatic environments. The solubility of these Fe(III) (hydr)oxides depends greatly on the pH of the environment, ionic strength, and the presence of organic ligands. Goethite (α -FeOOH) and hematite (Fe_2O_3) are the most common Fe(III) (hydr)oxide found in soils (Kraemer, 2004). Goethite can be present in both aerobic and anaerobic soils while hematite is mostly found in aerobic soils. Ferrihydrite, although less commonly found in soils, can possibly contribute to soluble iron concentrations due to its high solubility and surface area. Hematite and goethite are considered more stable than ferrihydrite; however, as particle sizes decrease, the solubility of hematite and goethite increases approaching ferrihydrite (Kraemer, 2004). Typical particle diameters of hematite and goethite found in soil range from 10 to 150 nm (Cornell and Schwertmann, 2003). The solubility of Fe(III) (hydr)oxides is also strongly influenced by the pH of the solution, where minimum solubilities are seen at

neutral to alkaline pH values (Kraemer, 2004). However, many chelators such as siderophores have a significant effect on Fe(III) (hydr)oxide solubilities.

Role in U Redox Reactions

Extensive research has been devoted to studying U reduction and oxidation; however, until recently, few have studied U redox reactions in the presence of soil minerals. The Eh-pH diagram for U is shown in Figure 1.1 in the presence and absence of bicarbonate. It has been shown that Fe(II) and Fe(III) play important roles in the abiotic U reduction and oxidation by the following equation (Sani et al., 2005):

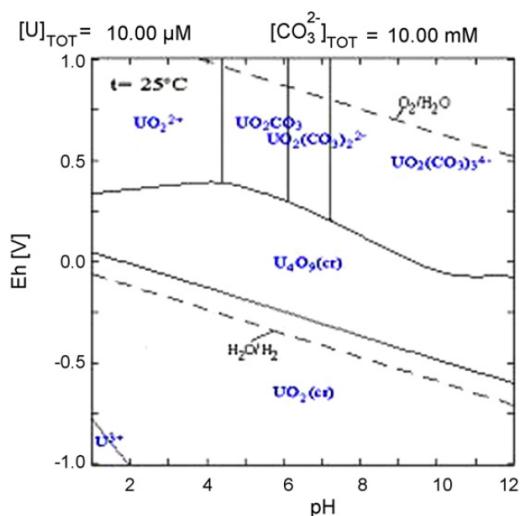
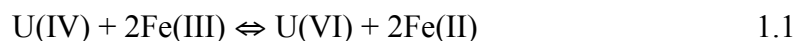


Figure 1.1a

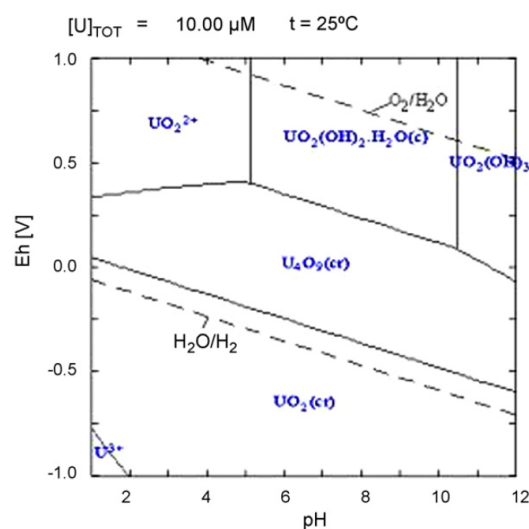


Figure 1.1b

Figure 1.1. Eh-pH diagram for uranium with carbonate (a) and without carbonate present (b) (Gavrilescu et al., 2009).

The presence of bicarbonate in the soil will shift Equation 1.1 to the right due to U(VI) binding to bicarbonate to form stable complexes. Bicarbonate increases U(VI) solution concentrations by extracting U(VI) from solid surfaces and pulling into solution to form complexes. High bicarbonate concentrations also limit UO_2 surface passivation allowing for an increased rate of UO_2 oxidation by ferrihydrite, which likely proceeds through a soluble U(IV) intermediate adsorbing onto ferrihydrite followed by electron transfer (Ginder-Vogel et al., 2010). Table 1.1 lists the complete reactions for the Fe(III) (hydr)oxides with UO_2 .

Table 1.1. Table of redox reactions for UO_2 and ferrihydrite ($\text{Fe}(\text{OH})_3$), goethite (FeOOH), or hematite (Fe_2O_3) in the presence and absence of bicarbonate.

Redox Reaction	Equation Number
$2\text{Fe}(\text{OH})_3 + 4\text{H}^+ + \text{UO}_2 + 0.5\text{HCO}_3^- \rightarrow 2\text{Fe}^{2+} + 4.5\text{H}_2\text{O} + 0.5(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$	1.2 ^a
$2\text{FeOOH} + \text{UO}_2 + 4\text{H}^+ + 0.5\text{HCO}_3^- \rightarrow 0.5(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^- + 2\text{Fe}^{2+} + 2.5\text{H}_2\text{O}$	1.3 ^a
$\text{Fe}_2\text{O}_3 + \text{UO}_2 + 4\text{H}^+ + 0.5\text{HCO}_3^- \rightarrow 0.5(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^- + 2\text{Fe}^{2+} + 1.5\text{H}_2\text{O}$	1.4 ^a
$2\text{Fe}(\text{OH})_3 + 6\text{H}^+ + \text{UO}_2 \rightarrow \text{UO}_2^{2+} + 2\text{Fe}^{2+} + 6\text{H}_2\text{O}$	1.5
$2\text{FeOOH} + \text{UO}_2 + 6\text{H}^+ \rightarrow \text{UO}_2^{2+} + 2\text{Fe}^{2+} + 4\text{H}_2\text{O}$	1.6
$\text{Fe}_2\text{O}_3 + \text{UO}_2 + 6\text{H}^+ \rightarrow \text{UO}_2^{2+} + 2\text{Fe}^{2+} + 3\text{H}_2\text{O}$	1.7 ^b

a cited from Guillaumont et al. (2003)

b cited from Nevin and Lovley (2000)

Previous abiotic experiments have shown that aqueous Fe(III) can rapidly and completely reoxidize chemically synthesized UO_2 in the presence of bicarbonate while Fe(III) added as hematite did not lead to reoxidation during the course of the experiment

(Sani et al., 2005). Studies using pure cultures of *D. desulfuricans* G20 demonstrated partial reoxidation of biogenic UO_2 with the addition of a Fe(III) (hydr)oxide (hematite, goethite, or ferrihydrite). The amount of UO_2 reoxidized increased with increasing amounts of hematite added when grown under electron-donor-limited sulfate-reducing conditions. Biogenic UO_2 was suggested as the electron donor for Fe(III) reduction with hematite being the most effective Fe(III) source compared to goethite and ferrihydrite (Sani et al., 2005). Using thermodynamic models, Ginder-Vogel et al. (2006) demonstrated ferrihydrite reoxidation of UO_2 is favorable under certain conditions while hematite and goethite are less favorable for UO_2 reoxidation (see chemical equations in Table 1.1).

Previous studies with siderophores, especially DFB, have demonstrated the ability of siderophores to promote dissolution of goethite and hematite (Hersman et al., 1995; Cheah et al., 2003; Reichard et al., 2007). The dissolution rates of goethite are one order of magnitude higher with DFB compared to the synthetic chelator, EDTA (Kraemer, 2004). Since ferrihydrite and hematite have previously been shown to have an impact on biotic UO_2 reoxidation (Sani et al., 2005; Ginder-Vogel et al., 2006), organic compounds capable of promoting Fe(III) (hydr)oxide dissolution could affect the stability of UO_2 through increasing soluble Fe(III) concentrations.

Chelators

Chelators are low molecular weight organic molecules that can bind to heavy metals forming stable complexes. Chelators can be made synthetically such as EDTA and NTA, while others are microbially produced including the siderophores, DFB and

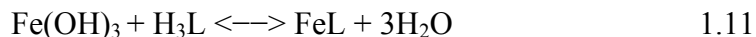
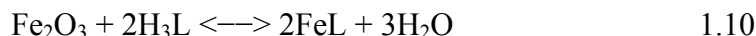
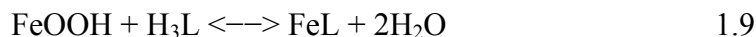
enterobactin. Additionally, microbial respiration products, such as citrate and pyruvate, can function as chelators. Chelators are categorized according to their denticities.

Denticity refers to the number of atoms of the chelator that bind to a central atom, or heavy metal, in the coordination complex. When only one atom of the ligand binds to a metal, the ligand is referred to as monodentate. Polydentate binding is when more than one atom binds to the metal. Complexes with polydentate ligands are generally more stable than those with monodentate ligands. The efficiency of the chelator for metal complexation depends on the number of available sites for metal fixation as well as the strength of the complex, which is expressed by the thermodynamic stability constant, $\log K$ (Equation 1.8). The larger the $\log K$ value, the higher the proportion of the metal-ligand (ML) complex exists when equal molar amounts of metal and the chelator are present (Duquene et al., 2008).

$$\log K = \log \left(\frac{[ML]}{[M][L]} \right) \quad 1.8$$

Equation 1.8 is based on a 1:1 complex of metal (M) to ligand (L)

Fe(III) chelators are known for their ability to solubilize Fe(III) minerals and Equations 1.9 to 1.11 represent the dissolution reactions of goethite, hematite, and ferrihydrite, respectively, in the presence of a chelator (H_3L) (Kraemer, 2004).



Citrate

Citrate (Figure 1.2) is abundant in nature due to continual production by microorganisms, and is found in plant roots and in the rhizosphere due to the decomposition of organic soil matter (Kantar et al., 2005). Rhizosphere citrate concentrations are estimated to be between 10 μ M and 100 μ M (Jones, 1998). Citrate has been found in radioactive waste sites (Choppin et al., 1996) and has been shown to bind to Fe(III) forming a 1:1 complex (Figure 1.3) with the stability constant $\log K = 11.19$ (NIST, 2004). Citrate can also form a bidentate structure with Fe(III); however, this stability constant is much lower ($\log K = 1.9-2.6$) (Francis et al., 1992). Citrate can also bind to U(VI) with a $\log K = 7.4$ (Table 1.2) with one proposed structure shown in Figure 1.4 where citrate binds to U(VI) with two atoms (NIST, 2004; Gavrilescu et al., 2009).

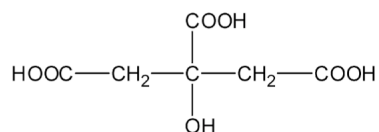


Figure 1.2: The structure of citric acid (Gavrilescu et al., 2009).

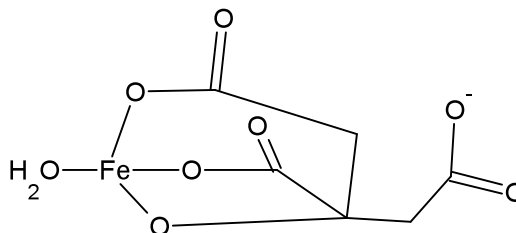


Figure 1.3: The structure of citric acid chelated with Fe(III) (Francis et al., 1992).

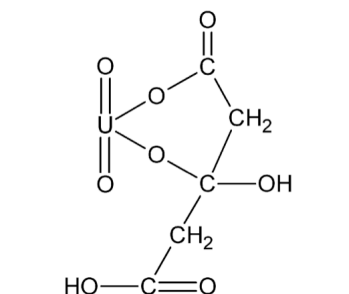


Figure 1.4. The structure of citric acid chelated with U(VI) (Gavrilescu et al., 2009).

Citrate has been shown to affect the microbial reduction of U(VI). Ganesh et al. (1997) demonstrated that in the presence of multidentate aliphatic ligands such as citrate, U(VI) reduction rate by the SRB, *D. desulfuricans* was decreased. However, rates of U(VI) reduction by the iron-reducing bacterium, *Shewanella algae*, were not impacted by citrate (Ganesh et al., 1997). Suzuki et al. (2010) also showed that in the presence of citrate, U(VI) reduction by the ferric-iron-reducing bacterium, *Shewanella putrefaciens*, was suppressed compared to the synthetic chelators NTA and EDTA. One possible explanation suggested the formation of polynuclear U(VI)-citrate complexes which would explain the slower reduction rate compared to EDTA and NTA. EDTA and NTA form mononuclear complexes with U(VI) despite EDTA and NTA having higher stability constants with U(VI) than citrate (log K values for EDTA, NTA, and citrate for U(VI) are 9.28, 9.5, and 7.4, respectively) (NIST, 2004). At neutral pH, U(VI) forms polynuclear complexes with citrate, such as $(\text{UO}_2)_3\text{Cit}_3^{3-}$ and $(\text{UO}_2)_6\text{Cit}_6(\text{OH})_{10}^{16-}$ (Pasilis and Pemberton, 2003). Since citrate can bind strongly to U(VI), and has been shown to impede the reduction of U(VI) to U(IV) by bacteria, this ligand influences stability and mobility of U; however, much more research is needed to truly understand citrate's role in U subsurface fate.

EDTA and NTA

EDTA (Figure 1.5a) is synthetically made and is widely used in industry. EDTA is characterized as a hexadentate metal chelator that can bind strongly with Fe(III) forming a 1:1 complex (Figure 1.7) with a stability constant of log $K = 25$ (Winkelmann, 1991; Bucheli-Witschel and Egli, 2001). Similar to some actinides, EDTA forms a 1:1

complex with U(VI) (one proposed structure shown in Figure 1.6) with a stability constant of $\log K = 9.28$ (Table 1.2) (NIST, 2004; Gavrilesco et al., 2009). NTA shown in Figure 1.5b is also synthetically made, but considered more easily biodegradable than EDTA and is tetradentate, or has four binding sites available for chelation. NTA binds strongly forming a 1:1 complex with both Fe(III) (Figure 1.9) and U(VI) (Figure 1.8) ($\log K$ values are 16 and 9.56, respectively) although it does not bind as strongly as EDTA (Table 1.1) (Gavrilesco et al., 2009; Suzuki et al., 2010).

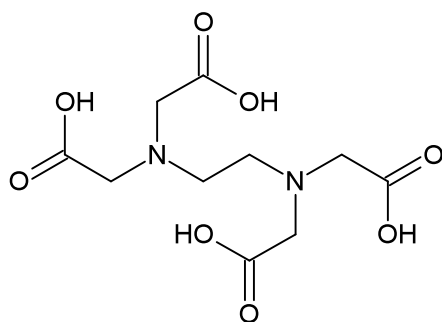


Figure 1.5a

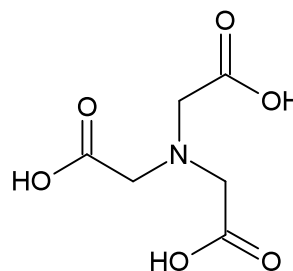


Figure 1.5b

Figure 1.5: Structure of EDTA (a) and NTA (b).

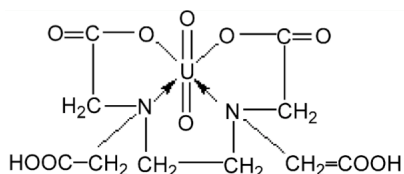


Figure 1.6: The structure of EDTA chelated with U(VI) (Gavrilesco et al., 2009).

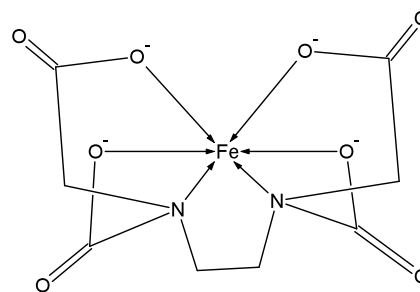


Figure 1.7: The structure of EDTA chelated with Fe(III) (Bucheli-Witschel and Egli, 2001).

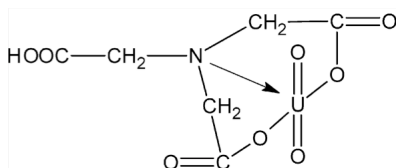


Figure 1.8: The structure of NTA chelated with U(VI) (Gavrilescu et al., 2009)

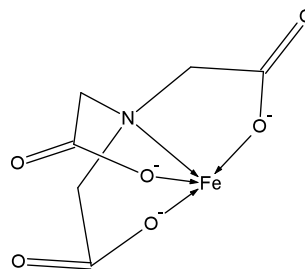


Figure 1.9: The proposed structure of NTA chelated with Fe(III)

EDTA and NTA are considered aminopolycarboxylic acids or APCAs and are both abundant in soil and groundwater with EDTA being one of the most studied anthropogenic complexing agents. The highest environmental concentrations of APCAs were reported at the Hanford site, USA, where both complexing agents and radioactive metals are found (Bucheli-Witschel and Egli, 2001). EDTA is persistent in soils and one reported half-life was 6 months (Means et al., 1980); however, EDTA bound to Fe(III) in river water had one reported half-life of 20 days (Bucheli-Witschel and Egli, 2001). NTA is less persistent on soil and a half-life of 3 to 7 days was reported under aerobic conditions (Duquene et al., 2008).

EDTA and NTA have been shown by Suzuki et al. (2010) to inhibit the reduction of U(VI) to U(IV) by *S. putrefaciens* compared to the no-chelator control by forming a mononuclear complex with U(VI) and no reductive UO_2 precipitation occurred due to the formation of stable U(IV)-organic complexes. Thus, these synthetic chelators are considered problematic for remediation purposes due to the formation of stable soluble complexes, which enhance U mobility and also prevent formation of insoluble biogenic UO_2 .

Siderophores

Siderophores are defined as low molecular weight (500-1000 Daltons) chelating molecules with a very high affinity for iron, specifically Fe(III). Aerobic and anaerobic bacteria and fungi produce siderophores. They are typically secreted into the extracellular environment when low iron concentrations occur. Their role is to scavenge iron from the environment making it more bioavailable.

More than 500 different siderophores have been described to date. Siderophores have a strong affinity for Fe(III) typically forming a 1:1 complex with a log K value > 30 (Boukhalfa and Crumbliss, 2002). The majority of siderophores are hexadentate ligands although microbes also produce tetradentate and bidentate siderophores and two or three molecules of the ligand assemble to satisfy the six coordination sites of iron. The denticity of siderophores plays an important role in determining its affinity for Fe(III) or Fe(II) or both. Generally, the higher the denticity, the greater the affinity towards iron where hexadentate siderophores have a higher affinity to iron compared to siderophores with a lower number of iron binding functional groups (Boukhalfa and Crumbliss, 2002). Cyclic siderophores such as desferrioxamine E have a higher affinity towards Fe(III) compared to the similar linear siderophore, DFB. Thus, both denticity and ligand architecture play an important role in metal chelation by siderophores.

Desferrioxamine B

DFB, shown in Figure 1.10, a hexadentate bacterial siderophore that is excreted by *Actinomycetes*, binds strongly in a 1:1 ratio with Fe(III) with the log $K = 30.6$ (Figure 1.11) (Winkelmann, 1991). DFB is secreted by the bacterium to scavenge iron and is

vital for the survival of bacteria. DFB also has the ability to bind to other metals such as aluminum (III) and calcium (II); however, the stability constants with these metals are many orders of magnitude less when compared to Fe(III) (Kraemer, 2004). DFB, as well as other siderophores, have been shown to promote dissolution of iron-bearing minerals including hematite and goethite (Hersman et al., 1995; Kraemer et al., 1999; Cheah et al., 2003; Reichard et al., 2007). Soil concentrations of hydroxamate siderophores have been estimated to be between 10^{-7} and 10^{-8} M (Powell et al., 1980). With the help of iron chelators such as DFB, the amount of soluble iron should also increase, with the amount dependent on chelator type and concentration.

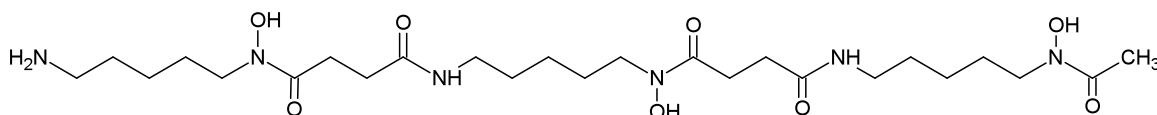


Figure 1.10: The structure of DFB

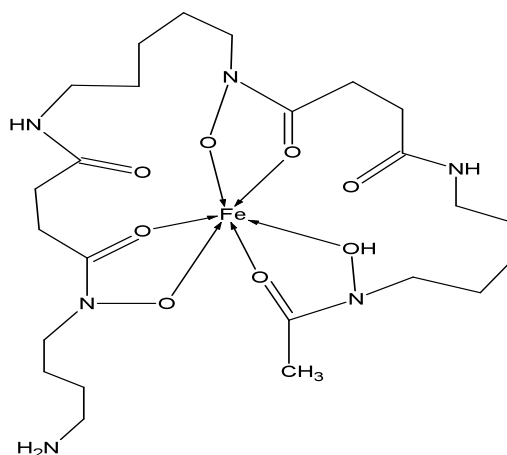


Figure 1.11: The structure of DFB bound to Fe(III) (Siebner-Freibach et al., 2004).

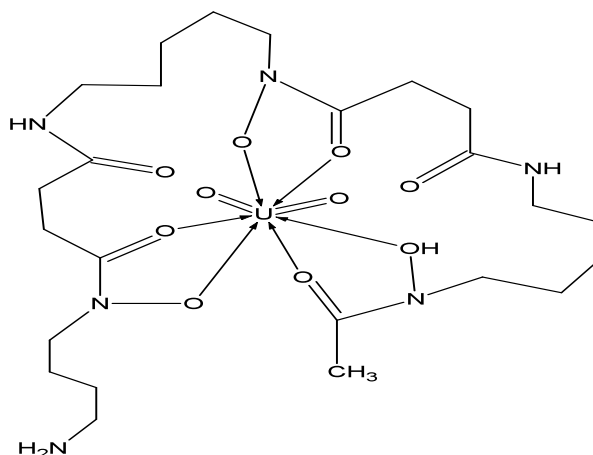


Figure 1.12: The proposed structure of DFB bound to U(VI) (Gavrilescu et al., 2009).

DFB has also been shown to bind to tetravalent actinides forming 1:1 complexes with high stability constants ($\log K = 30.8$ for Plutonium(IV) and 26.6 for Thorium(IV)) (Hernlem et al., 1999). As the stability constants are similar between DFB with Fe(III) and actinides, the potential for competition for complexation exists. DFB binds to U(VI) (the proposed structure shown in Figure 1.12) with a stability constant value of $\log K = 17.12$ at pH 7 (see Table 1.2) with the major structure present being UO_2DFBH (Mullen et al., 2007; Gavrilescu et al., 2009). DFB has experimentally been shown to promote dissolution of UO_2 and thus could also play an important role in U stability (Brainard et al., 1992; Frazier et al., 2005).

Table 1.2. Stability constants of Citrate, DFB, EDTA, and NTA for various metals with an ionic strength of 0.1 at 25°C or where indicated (^a), at 20°C (NIST, 2004)

Metal Ion	Log $K_{\text{MeCitrate}}$	Log K_{MeDFB}	Log K_{MeEDTA}	Log K_{MeNTA}
Fe(III)	11.19	30.76 ^a	25.1	16
Fe(II)	4.8	-	14.3	8.9
U(VI)	7.4	17.12 ⁽¹⁾	9.28	9.5
U(IV)	-	-	25.7	-

(1) cited from Mullen et al., 2007

Summary

DFB has previously been shown to promote dissolution of chemically synthesized UO_2 and Fe(III) (hydr)oxides (Kraemer, 2004; Frazier et al., 2005). The current investigation examined the effects of DFB on biogenic UO_2 reoxidation, as well as the possible mechanism of UO_2 reoxidation. The stability constants for all chelators and metals, Fe(III) , U(IV) , and U(VI) , were taken into consideration when developing trends. Citrate, EDTA, and NTA have all previously been shown to inhibit bacterial U(VI) reduction (Suzuki et al., 2010), so their role in U(IV) oxidation was investigated. Different Fe(III) (hydr)oxides were used in these experiments including ferrihydrite, goethite, and hematite, and their affect on U stability was examined in the presence and absence of the chelator. Bicarbonate has been shown to have environmental importance with U and influence on the reoxidation of UO_2 by Fe(III) , so bicarbonate was also examined. A look at these experiments gained insight into U stability and what factors that are abundant in nature will need to be monitored to ensure effective long-term U immobilization as well as aid in bioremediation efforts.

CHAPTER 2

METHODS

Experimental Goals

Metal complexation agents, ligands, and chelators are potentially problematic for U remediation efforts, due to complex formation enhancing U and iron solubility. These chelators can affect the stability of U and may be undesirable for the bioremediation of U at contaminated sites. This chapter describes the methods used to determine whether chelators, DFB, citrate, EDTA, or NTA, affected reoxidation rates of biogenic UO_2 by Fe(III). The rate-limiting step was determined by examining dissolution rates of biogenic UO_2 , ferrihydrite, goethite, and hematite with and without a chelator present. Control experiments were conducted and consisted of iron-free and uranium-free controls.

Materials and MethodsMaterials

All glassware was soaked in a 0.5M HCl bath for at least 2 hours prior to use. 95% DFB was purchased from Sigma, >99% sodium citrate (Fisher), EDTA disodium salt (> 99.5%) was purchased from Fisher, and NTA trisodium salt monohydrate (99+ %) was purchased from Acros Organics. Stock solutions (10mM) of DFB were used within a month, while sodium citrate, EDTA, and NTA stock solutions were made prior to each experiment. U standard solution in 5% nitric acid (1000ppm +/- 10 $\mu\text{g/mL}$) was purchased from GFS Chemicals, Inc. (Columbus, OH). Iron reference standard solution

(1000ppm +/- 1%) was purchased from Fisher Chemical in the form of ferric nitrate, nonahydrate in 2% nitric acid. The preparation of biogenic UO_2 as well as sampling and assembling of all experiments took place in an anaerobic chamber (Coy Laboratory Products Inc.) containing a pre-mixed gas of 90% N_2 , 5% H_2 , and 5% CO_2 (by volume) that was circulated through a Palladium catalyst and silica gel. The O_2 concentration was monitored with a Coy oxygen meter and maintained below 1ppm.

Biogenic UO_2 was made as described by Ulrich et al. (2008) and produced by *Shewanella* CN32 (CN32) through the reduction of uranyl chloride (UO_2Cl_2) coupled to lactate oxidation under anaerobic conditions at pH 7. Medium components consisted of the following per 1L: 30mM KHCO_3 , 1.5 μL Wolfe's minerals, 10mM PIPES, 1.375g UO_2Cl_2 , 0.005g KCl, 0.05g MgSO_4 , 0.03g NaCl, 1g NH_4Cl , 1g KH_2PO_4 , and 30mM sodium lactate. All medium components were added together, with UO_2Cl_2 added last, and the pH was adjusted to 7. The medium was then autoclaved, degassed with N_2 , and stored in the glovebag until usage.

CN32 cells were initially grown in the medium without UO_2Cl_2 . During the exponential phase, CN32 was centrifuged and the pellet was resuspended in KHCO_3 buffer, re-centrifuged, and resuspended in medium without UO_2Cl_2 . This CN32 slurry was then added to a 1L reactor with all medium components mixing at 130rpm and stored in the glovebag during biotic U(VI) reduction. After 5 days, 15mM of sodium lactate was added to the reactor to ensure the reduction of any excess UO_2Cl_2 . After 12 days total, the reactor was shut down and the suspension was centrifuged in anaerobic centrifuge bottles at 6500rpm (6890 x g) for 20 minutes. 250mL of 1M NaOH was added

to the pellet and shaken for 5 minutes to destroy any intact cells and centrifuged again at 6500rpm for 20 minutes, then decanted. 250mL of anoxic nanopure water was added to the pellet, shaken for a minute, centrifuged at 6500rpm for 20 minutes, decanted, and resuspended in 250mL of anoxic nanopure water. Approximately 100mL of anaerobic hexane was added to the culture in the glovebag and mixed forming two distinct layers. The organic layer containing the biological substances was separated from the reduced U using a separation funnel. The reduced U and water layer was then centrifuged for 20 minutes at 6500rpm and decanted. The reduced U was resuspended in 100mM anaerobic KHCO_3 in the glovebag while being stirred to remove excess aqueous U(VI) adsorbed on the particles. After 2 days, the UO_2 was centrifuged for 20 minutes at 6500rpm, decanted, and resuspended in anoxic water. The biogenic UO_2 was stored in the anaerobic chamber until use and the UO_2 concentration was measured by oxidizing unfiltered samples with concentrated HNO_3 overnight and analyzing on the kinetic phosphorescence analyzer, or KPA-11 (as described below).

Synthesis of Iron (hydr)oxide-Coated Sands

Ferrihydrite was prepared as described by Brooks et al. (1996). 6.39g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Spectrum Chemical Mfg. Corp., Gardena, CA) was dissolved in 383mL of nanopure water. Then 0.4N NaOH was slowly added, 1mL at a time, to the ferric chloride solution while being continuously stirred until the pH reached 7.5 and remained stable over 1 hour. The ferrihydrite solution then sat until two layers formed with the ferrihydrite slurry on the bottom, and the salt layer on top, which was then decanted after sitting overnight. Nanopure water was added to the slurry until the volume reached

400mL. This solution was divided into two 250 mL centrifuge bottles and centrifuged at 6000rpm (5870 x g) for 15min. The supernatant was decanted and the pellet was resuspended in nanopure water. This centrifuge and resuspension sequence was repeated for a total of three times. After the third centrifugation, the Fe solution was combined into one bottle, centrifuged one last time for 15 minutes at 6000rpm, decanted, and poured over quartz sands.

Goethite was prepared using a modified method as described by Atkinson et al. (1967). A ferric nitrate solution was prepared and NaOH was added, 1 mL at a time, to this solution while being continuously stirred over 3 to 4 hours until a pH of 12 was reached. This iron slurry was placed into a 60°C oven for 24 hours. Afterwards, the salts were removed by dialysis tubing placed into a plastic tray with nanopure water during a period of approximately two weeks. The goethite slurry was stored at 4°C until needed to coat the quartz sand.

Hematite was prepared following the method described by Schwertmann and Cornell (2000). 5L of distilled water were brought to a boil while continuously stirred. Then 400mL of 1M ferric nitrate was slowly added over a period of 4 hours to the boiling water. This solution was then cooled overnight and the salts were removed by dialysis tubing placed in plastic trays with nanopure water for approximately two weeks. The hematite slurry was then stored at 4°C until needed to coat the quartz sand.

Each Fe(III) (hydr)oxide sample was prepared individually. Each iron slurry was poured onto quartz sand (Iota 6 Quartz Sand, Umimin Corp.) in separate plastic trays and mixed by hand approximately every 4 hours until dried. For ferrihydrite, all of the

ferrihydrite slurry was added to 110g of sand. For hematite and goethite, the amount of slurry added to the sand was added to make the same consistency as ferrihydrite (approximately 20mL of slurry added to 110g of sand). The sands were allowed to dry in a fume hood for at least 48 hours. After that period of time, the iron-coated sands were rinsed with nanopure water by gentle rotation of the tray and the excess water was poured off. This was repeated 20 to 30 times or until the water being poured off was clear. After rinsing, the iron-coated sands were allowed to dry for at least 12 hours in the fume hood. Hematite and goethite were stored at room temperature until needed while ferrihydrite was stored at room temperature and used within 2 weeks of preparation.

Fe(III) concentrations on the sand were measured by adding 1mL of concentrated HNO_3 to 0.1g of iron-coated sand to allow Fe(III) to dissociate from the quartz sand. Aqueous Fe(III) concentrations were measured on the inductively coupled plasma mass spectrometer (ICP-MS) to determine the amount of Fe(III) per gram of sand. Fe(III) concentrations were roughly 0.007g of Fe(III) per gram of sand.

Analytical Methods

Aqueous U(VI): To measure aqueous U(VI) concentrations, 0.3mL of sample was pulled by syringe in the anaerobic chamber from each bottle and filtered (0.2 μm , Fisher). 15 μL of the filtered sample was placed into a scintillation vial (Fisher), which was acid washed in 1M HNO_3 (Fisher) bath prior to use, and the lid was tightly fastened to prevent air entering the vial. Samples were taken out of the glovebag in small groups to ensure no UO_2 oxidation due to oxygen occurred. 3mL of 0.1M H_2SO_4 (Fisher) were added to the sample in the vial and mixed. Then 1.333mL of this solution was added to

2mL of dilute uraplex (Chemcheck Instruments Inc.) in a glass cuvette and placed manually in the KPA-11 (Chemcheck Instruments Inc., Richland, WA). Calibration curves for the KPA-11 were made with a U standard solution diluted in 0.1M H₂SO₄ ranging from concentration values of 0μM to 150μM. Both samples and standards were diluted 200 times. All negative KPA-11 responses were regarded as zero U(VI) concentrations and lowest detection limit was approximately 0.5μM.

Total Dissolved U: To measure total dissolved U concentrations, 0.3mL of sample were filtered (0.2μm, Fisher) and then 0.2mL of supernatant was placed into a 3KDa Nanosep centrifugal device (Pall Life Sciences) in the anaerobic chamber and centrifuged at 14,000rpm (14,565 RCF) outside the anaerobic chamber for 15min to eliminate any solid U that may have passed through the 0.2μm filters. 0.1mL of this filtered sample was added to 0.3mL of concentrated HNO₃, vortexed, and allowed to sit for at least 1 hour to allow any dissolved U(IV) to oxidize to U(VI). Then the samples were measured on the KPA-11 by adding 15μL of this concentrated solution to 2.37mL DI water and 0.63mL of 0.1M HNO₃ in a scintillation vial. After mixing, the sample vials were placed in the rack to be analyzed by the KPA-11 on automatic mode. Total U was measured and soluble U(IV) concentrations were calculated by difference. A separate calibration curve was used for total U measurements with the U standard diluted in 0.1M HNO₃ at concentrations ranging from 0μM to 150μM. All negative KPA-11 responses were regarded as zero total U concentrations and lowest detection limit was approximately 0.5μM.

Total Dissolved Iron: Soluble iron concentrations were measured by ICP-MS.

Bottles were brought into the glovebag for sampling and 2mL of sample were pulled and filtered through 0.2 μ m filters. Samples were diluted in a 5% HNO₃ (Optima grade, Fisher) matrix by taking 1.7mL of the filtered sample and adding to 3.3mL of 7.6% HNO₃ (Optima grade, Fisher) and stored at 4°C until analysis on the ICP-MS. Iron standards in 5% HNO₃ were made using the iron reference standard solution at concentrations ranging from 0ppm to 50ppm with the lowest detection limit for iron of approximately 0.01ppm.

Dissolution Experiments of Fe(III) (hydr)oxide in the Presence of Chelators

To investigate the effects of various chelators on dissolution of Fe(III) (hydr)oxides, batch experiments were set up containing 100mL of 10mM PIPES (1,4-Piperazinediethanesulfonic acid, Sigma) buffer at pH 7 with 2g of iron-coated sands (about 2.5mM Fe(III) total in each serum bottle) with either 0mM chelator (control), 0.1mM chelator, or 0.2mM chelator (DFB, citrate, EDTA, or NTA). In the experiments with EDTA and NTA used as the chelator, potassium bicarbonate (Fisher) was also added to obtain a final concentration of 10mM. 10mM PIPES buffer was prepared in advance in serum bottles, autoclaved and immediately made anoxic after autoclaving by cooling under a stream of N₂ gas, and finally stored in the anaerobic chamber. Stock solutions of each chelator were made individually using anoxic water made by autoclaving nanopure water and cooling under a stream of N₂ gas and stored in the glovebag. The batch systems were assembled and sealed inside the glovebag and then shaken outside the glovebag at room temperature in between samplings; bottles were brought into the

glovebag during sampling. Samples were filtered through 0.2 μ m filters and stored in 5% nitric acid at 4°C until ICP-MS analysis. Separate experiments were run for each of the three Fe(III) (hydr)oxides tested (ferrihydrite, goethite, and hematite) as well as each chelator tested (DFB, citrate, EDTA, and NTA). Experiments were conducted in triplicate and sample error values were calculated by standard deviation from the average. These experiments were referred to as uranium-free controls.

Dissolution Experiments of Biogenic UO₂ in the Presence of Chelators

To investigate dissolution rates of UO₂ by different chelators, serum bottles were set up containing biogenic UO₂ (125 μ M), 50mL of 10mM PIPES buffer at pH 7 with either no chelator, 0.1mM chelator, or 0.2mM chelator (DFB, citrate, EDTA, or NTA) with different potassium bicarbonate concentrations (0mM, 3mM, or 10mM). With EDTA and NTA, only the 10mM bicarbonate concentration was used. Systems with no chelators present were used as controls for these experiments. PIPES buffer and stock solutions of each chelator were prepared as described above. Batch systems were assembled in a glovebag and then shaken outside the glovebag at room temperature between samplings; bottles were brought into the glovebag during sampling. Samples were filtered through 0.2 μ m filters and analyzed for U(VI). Then the filtered sample was measured for total dissolved U to calculate aqueous U(IV) concentrations by subtraction. Separate experiments were run for each chelator tested (DFB, citrate, EDTA, and NTA). Experiments were conducted in triplicate and sample error bars represent standard deviation between triplicates. These experiments are referred to as iron-free controls.

UO₂ Reoxidation Experiments

Fe(III) (hydr)oxides: To investigate the effects of chelating agents on U(IV) reoxidation in the presence of Fe(III) (hydr)oxides, batch experiments were set up in the same way as the iron-free controls (described above) with the addition of 1g of iron-coated sands (about 2.5mM of Fe(III) in each serum bottle). Samples were filtered (0.2µm) and only measured for U(VI) concentrations using the KPA-11 over the course of approximately 80 days. A separate experiment was run for each of the three Fe(III) (hydr)oxides (ferrihydrite, goethite, and hematite) and the different chelators tested (DFB, citrate, EDTA, and NTA).

Ferric Chloride: To investigate the role of aqueous Fe(III) on the reoxidation of biogenic UO₂, batch bottles were set up containing 50mL of FeCl₃*6H₂O (25mM, 2.5mM, 1.25mM, and 0mM) made with anoxic water with approximately 1.25mM of UO₂. The pH values ranged from 2.2 to 3.1 and were not adjusted. A control system was set up with anoxic water with no ferric chloride at pH 2 with 1.25mM UO₂. The batch systems were assembled in the glovebag and then shaken outside the glovebag at room temperature in between samplings; bottles were brought into the glovebag during sampling. Samples were diluted 1:2000 with 0.1M H₂SO₄ and then measured for U(VI) using the KPA-11. Separate experiments were run for each different iron concentration and conducted in triplicate.

Fe(III) Bound to Chelator: To investigate how Fe(III) bound to a chelator will affect U(IV) reoxidation, serum bottles were set up containing either 50mL of 2.5mM

ferric citrate (Fisher) at pH 2.6, 5.4 and 7.1 with 1.25mM UO_2 or 50mL of ferric chloride bound to DFB with 1.25mM UO_2 . To make a solution of ferric chloride bound to DFB, ferric chloride was made with anoxic water in a separate container. DFB was then added to this container and allowed to sit for about 15 minutes to allow all of the DFB to bind to free Fe(III). Concentrations used were 1.25mM ferric chloride bound to 1.25mM DFB (pH of 2.1, 5, and 7.1) and 1.25mM ferric chloride bound to 0.625mM DFB (pH 2.5). Samples were diluted 1:2000 with 0.1M H_2SO_4 and then measured for U(VI) using the KPA-11. Separate experiments were run for each different chelator bound to Fe(III) as well as each different concentration and pH value. Each experiment was conducted in triplicate.

Rate Constant Calculations

Initial rate constants were calculated based on Equation 2.1 for zero-order reactions and 2.2 for non-zero-order reactions. Reaction order was determined by data and assumed to be zero-order if the rate was independent of the concentration of the chelator present. If greater concentrations of chelator present changed rate constants, then the reaction was assumed to be non-zero-order. Rate constants, k , were calculated using the slope of a graph of concentration versus time and only initial rates were used. Changes in volume were negligible and therefore, not accounted for in calculating rate constants. All rate constants calculated were either of zero or non-zero-order. For the dissolution experiments, rates were calculated based on solubilization since adsorption was not measured.

$$\frac{dU}{dt} = k_0 \quad \text{units on } k_0 \left[\frac{\mu\text{mol}}{L * h} \right] \quad 2.1$$

$$\frac{dU}{dt} = k_1 * [\text{Chelator}] \quad \text{units on } k_1 \left[\frac{1}{h} \right] \quad 2.2$$

where k_0 or k_1 is the rate constant and $[\text{Chelator}]$ is the concentration of the chelator used

CHAPTER 3

RESULTS AND DISCUSSION

U stability is of primary concern for remediation strategies due to the many factors that play an important role in the subsurface fate of U. To identify the most effective remediation techniques, more research is needed to characterize U stability. The goal of this project was to quantify the effects of different iron chelators and iron minerals on bio-reduced U and identify the rate and extent of reoxidation.

Dissolution of UO_2 with Iron MineralsDissolution Experiments with DFB

Figure 3.1 shows the time course of total dissolved iron in the DFB-free control, 0.1mM DFB, and 0.2mM DFB with ferrihydrite (Figure 3.1A), goethite (Figure 3.1B), or hematite (Figure 3.1C) at pH 7. In the DFB-free control, there was no dissolved iron observed; however, in all systems, DFB promoted dissolution of iron minerals. With ferrihydrite, there was a significant difference in dissolved iron concentration between the systems with 0.1mM and 0.2mM DFB (Figure 3.1A) and increased DFB concentrations increased aqueous iron resulting in a non-zero-order reaction (Table 3.1). With goethite and hematite, dissolved iron concentrations were approximately the same between 0.1mM and 0.2mM DFB (Figures 3.1B and C). This resulted in a zero-order reaction since DFB concentrations did not affect the rates of dissolution. Ferrihydrite was the most reactive of the iron minerals tested followed by hematite then goethite.

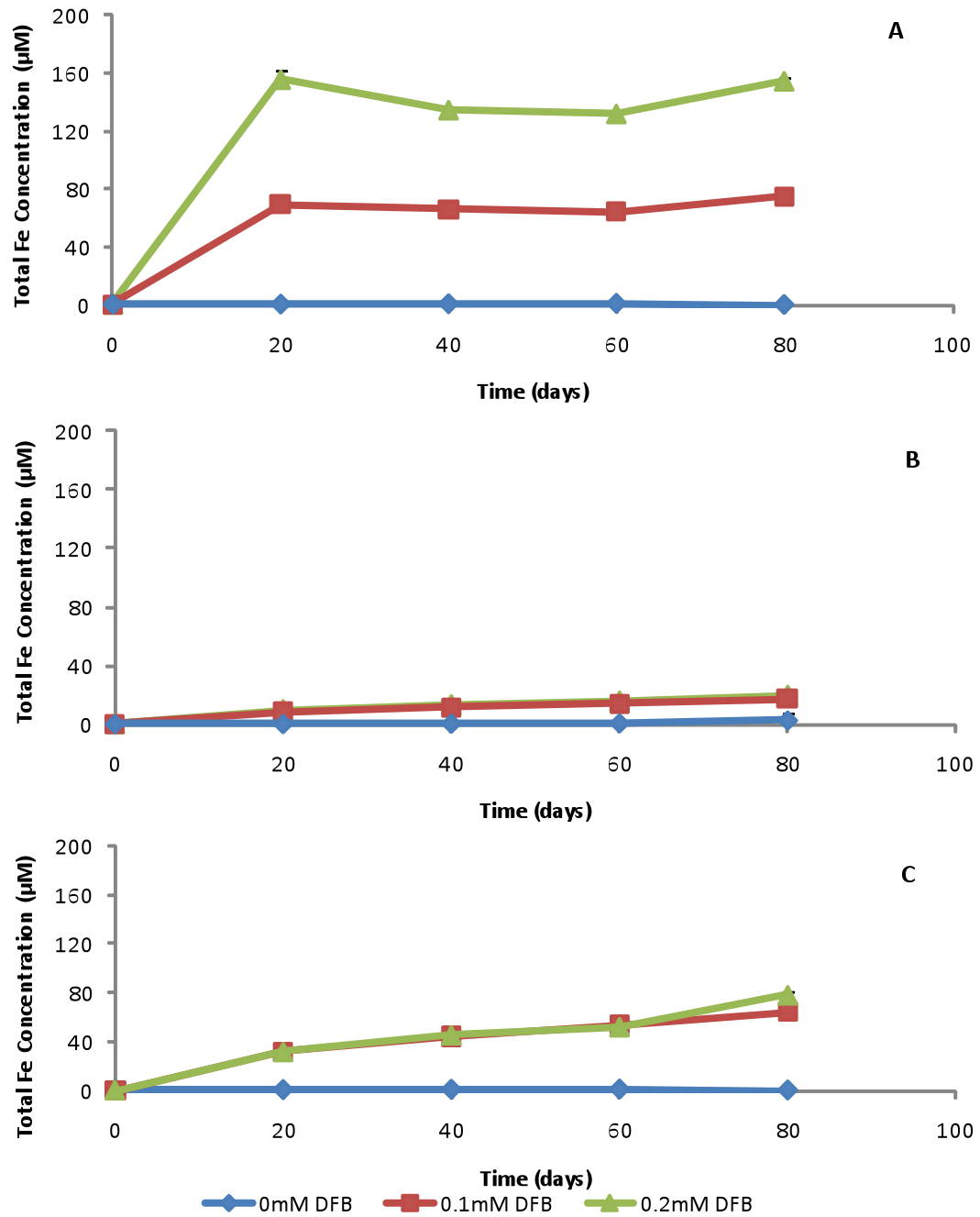


Figure 3.1. Dissolution of ferrihydrite (A), goethite (B), and hematite (C) by DFB over time showing DFB promotes dissolution. Error bars represent the standard deviation of the triplicates.

Table 3.1. Table of rate constant results from iron solubilization experiments by various chelators with ferrihydrite (Ferri), goethite (Goe), and hematite (Hem).

	Iron			
	Zero-Order k_0 [$\mu\text{mol Fe/ L*day}$]		Non-zero-Order k_1 [1/day]	
Iron Dissolution Experiments	Standard Deviation		Standard Deviation	
Ferri + DFB + 0Bi	--	--	3.7E-02 ± 2.8E-03	
Goe + DFB + 0Bi	2.1E-01 ± 1.4E-02		--	--
Hem + DFB + 0Bi	8.2E-01 ± 8.0E-02		--	--
Ferri + Cit + 0Bi	--	--	5.3E-04 ± 5.1E-04	
Goe + Cit + 0Bi	1.2E-01 ± 8.8E-02		--	--
Hem + Cit + 0Bi	2.9E-01 ± 3.1E-02		--	--
Ferri + EDTA + 0Bi	--	--	4.3E-02 ± 7.3E-04	
Ferri + EDTA + 10Bi	--	--	4.1E-02 ± 5.8E-04	
Goe + EDTA + 0Bi	1.7E+00 ± 2.6E-01		--	--
Goe + EDTA + 10Bi	6.0E-01 ± 1.7E-02		--	--
Hem + EDTA + 0Bi	5.3E+00 ± 1.2E+00		--	--
Hem + EDTA + 10Bi	2.0E+00 ± 3.5E-01		--	--
Ferri + NTA + 0Bi	--	--	4.3E-03 ± 1.6E-03	
Ferri + NTA + 10Bi	--	--	4.1E-03 ± 5.6E-04	
Goe + NTA + 0Bi	6.7E-03 ± 1.6E-02		--	--
Goe + NTA + 10Bi	2.2E-02 ± 5.3E-03		--	--
Hem + NTA + 0Bi	1.6E-02 ± 1.1E-02		--	--
Hem + NTA + 10Bi	2.6E-02 ± 3.8E-03		--	--

0Bi, 3Bi, and 10Bi are 0mM, 3mM, and 10mM bicarbonate concentrations
Cit = Citrate

Figure 3.2 shows dissolved U(VI) concentrations (Figures 3.2A and B) and total dissolved U (Figures 3.2C and D) concentrations as a function of time in the DFB-free control, 0.1mM DFB, and 0.2mM DFB at pH 7. In the DFB-free controls, dissolved U concentrations were always less than 2 μM . Only slight differences were observed between U(VI) values and total aqueous U in both the 3mM and 10mM bicarbonate, suggesting that most dissolved U was U(VI) and only a small fraction was U(IV) (< 2 μM). Figures 3.2A and B show the amount of DFB present affects U solubilization,

where 0.2mM DFB gave greater dissolved U(VI) than 0.1mM DFB leading to a non-zero-order reaction. However, since DFB is unable to oxidize UO_2 , these results suggest DFB extracted U(VI) adsorbed to UO_2 particles since initial solubilization rates (Table 3.2) between U(VI) and total U are very similar regardless of the amount of bicarbonate present ($k_l [\text{day}^{-1}] = 0.00032$ and 0.00055 or $k_l [\text{day}^{-1}] = 0.00034$ and 0.0006 for U(VI) and total U at 3mM bicarbonate or 10mM bicarbonate, respectively). There was also an increase in U(VI) with 10mM bicarbonate versus 3mM likely resulting from bicarbonate extraction of U(VI) adsorbed onto UO_2 .

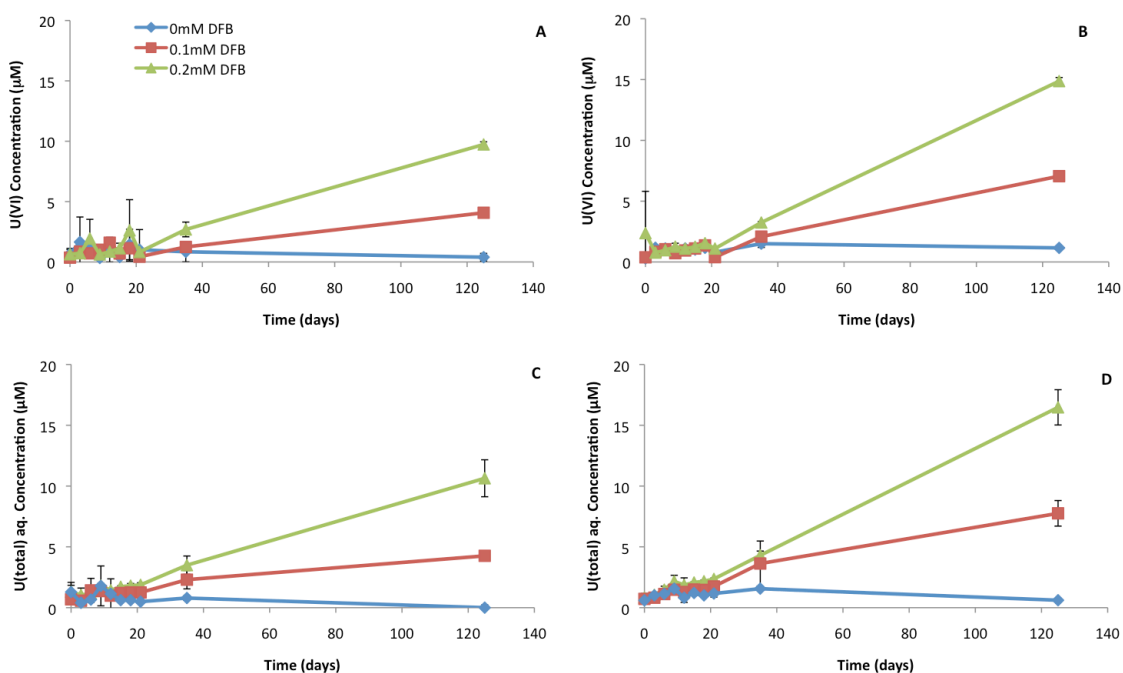


Figure 3.2. Dissolution of biogenic UO_2 by DFB with aqueous U(VI) concentrations at 3mM bicarbonate (A) and 10mM bicarbonate (B) showing DFB and bicarbonate increase the amount of soluble U(VI). Total soluble uranium concentrations at 3mM bicarbonate (C) and 10mM bicarbonate (D) showing DFB slightly increases aqueous U(VI) concentrations. Error bars represent the standard deviation from the triplicate's mean value.

Table 3.2. Table of rate constant results from U dissolution experiments for U(VI) and total dissolved U with various chelators and bicarbonate.

	U(VI)				U(Total) aq.			
	Zero-Order k_0 [μmol U(VI)/L*day]		Non-zero-Order k_1 [1/day]		Zero-Order k_0 [μmol Utotal/L*day]		Non-zero-Order k_1 [1/day]	
UO₂ Dissolution	Std. Dev.		Std. Dev.		Std. Dev.		Std. Dev.	
DFB + 3Bi	--	--	3.2E-04	$\pm 6.2\text{E-}05$	--	--	3.4E-04	$\pm 7.5\text{E-}05$
DFB + 10Bi	--	--	5.5E-04	$\pm 3.2\text{E-}05$	--	--	6.0E-04	$\pm 6.8\text{E-}05$
Cit + 0Bi	0	0	0	0	0	0	0	0
Cit + 3Bi	0	0	0	0	0	0	0	0
Cit + 10Bi	0	0	0	0	0	0	0	0
EDTA+10Bi	--	--	9.0E-04	$\pm 2.3\text{E-}04$	2.2E-01	$\pm 1.1\text{E-}01$	--	--
NTA + 10Bi	6.3E-02	$\pm 1.2\text{E-}02$	--	--	6.4E-02	$\pm 5.4\text{E-}02$	--	--

0Bi, 3Bi, and 10Bi are 0mM, 3mM, and 10mM bicarbonate concentrations

Standard Deviation is Std. Dev.

DFB shows only slight ability to increase the dissolution of UO₂ in these systems which contradicts the results shown by Frazier et al. (2005) which reported that DFB had very significant affects on the amount of U(IV) brought into solution compared to controls without DFB. One possible explanation is that their study used UO₂ that was synthesized chemically, whereas, biologically precipitated UO₂ was used here that could be less susceptible to reacting with chelators such as DFB. Also, our biogenic UO₂ was treated with bicarbonate washings to remove excess adsorbed U(VI), while the chemically precipitated UO₂ was not washed by bicarbonate but heated at 650°C for 7 hours to reduce excess U(VI) present. Previous studies (Bargar et al., 2008; Fletcher et al., 2010) have indicated the need for further research on the types of UO₂ formed abiotically versus biologically. UO₂ stability varies due to differences in particle size, the ability to form aggregates, and degree of crystallinity making it difficult to compare

results seen by biologically versus chemically precipitated UO_2 (Bargar et al., 2008; Fletcher et al., 2010).

Dissolution Experiments with Citrate

Figure 3.3 shows total aqueous iron versus time in the citrate-free control, 0.1mM, and 0.2mM sodium citrate with ferrihydrite (Figure 3.3A), goethite (Figure 3.3B), or hematite (Figure 3.3C), respectively, at pH 7. In the citrate-free controls, virtually no dissolved iron was detected during the course of the experiments. In all cases, 0.2mM citrate had greater dissolved iron concentrations than systems with 0.1mM citrate. Ferrihydrite was the most reactive, followed by hematite and then goethite, similar to DFB. However, when compared to the results with DFB, citrate is not as effective as DFB in solubilizing the iron minerals (Table 3.1), which can be expected since citrate has a much lower stability constant with iron than DFB (non-zero-order rate constant $k_I = 0.00053$ and 0.037 day^{-1} with ferrihydrite for citrate and DFB, respectively).

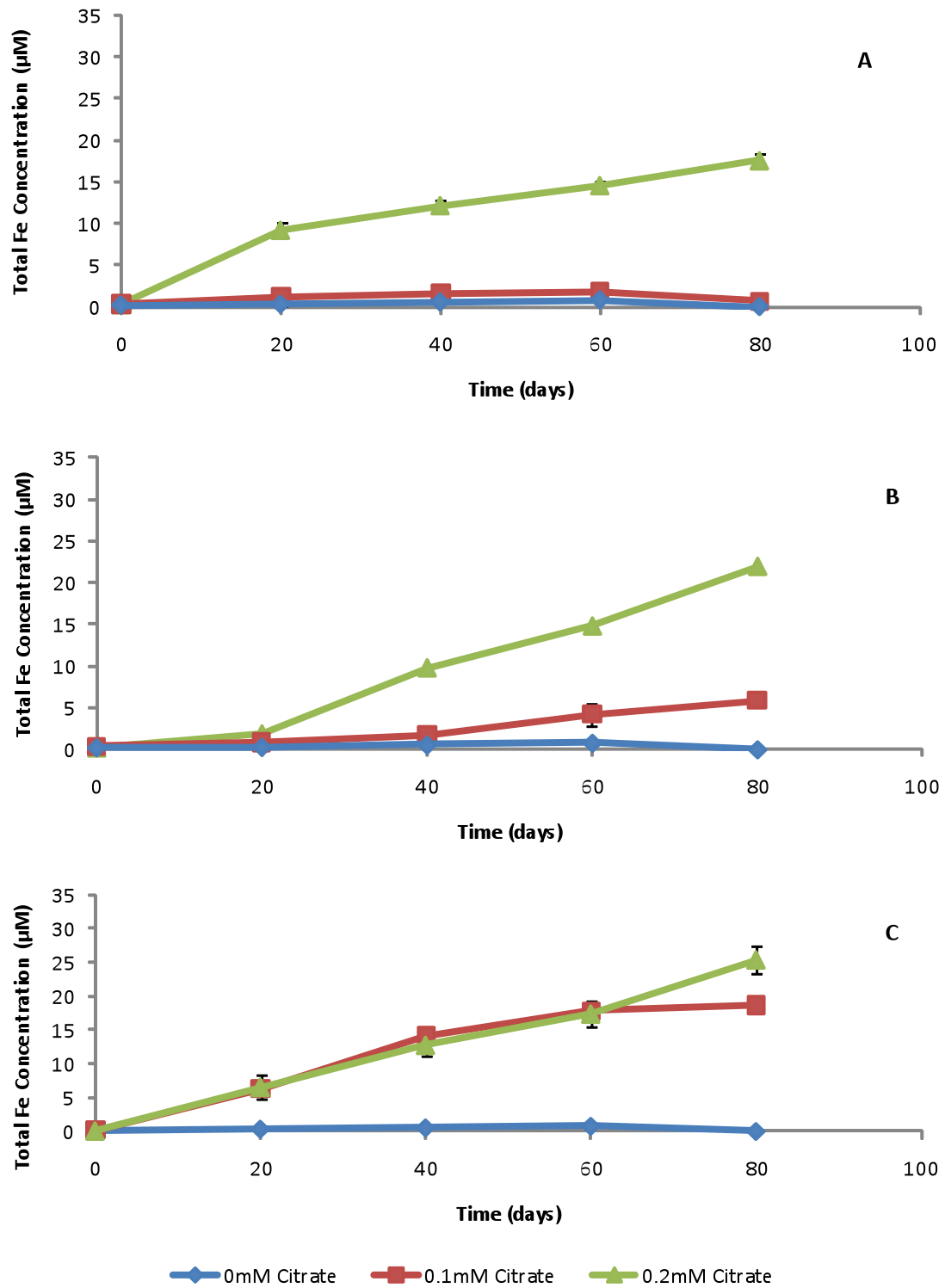


Figure 3.3. Dissolution of ferrihydrite (A), goethite (B), and hematite (C) by citrate showing citrate promotes dissolution. Error bars represent the standard deviation of the triplicates.

Figures 3.4 and 3.5 show the time courses of U(VI) (Figure 3.4) and total dissolved U (Figure 3.5) in the citrate-free control, 0.1mM, and 0.2mM sodium citrate. In all experiments, very little dissolved U(VI) ($< 2\mu\text{M}$) was detected and all rates measured were equal to zero (Table 3.1). There was virtually no difference between U(VI) concentrations and total dissolved U, suggesting that citrate was unable to promote UO_2 dissolution. In comparing dissolution of Fe(III) (hydr)oxides to dissolution of UO_2 , results show that citrate was solubilizing iron more quickly than UO_2 . After approximately 80 days, total aqueous U concentrations were $< 1\mu\text{M}$ while dissolved iron concentrations were $>10\mu\text{M}$ with goethite and $>20\mu\text{M}$ with ferrihydrite and hematite.

In the systems with 0.2mM citrate with goethite and 0.1mM citrate with hematite, contamination occurred resulting in inconsistent results amongst triplicates. Thus, the systems that were uncontaminated were graphed and error bars were removed. Contamination was observed in the serum bottles by the liquid being cloudy and confirmed under the microscope. Contamination only seemed to be an issue with these systems.

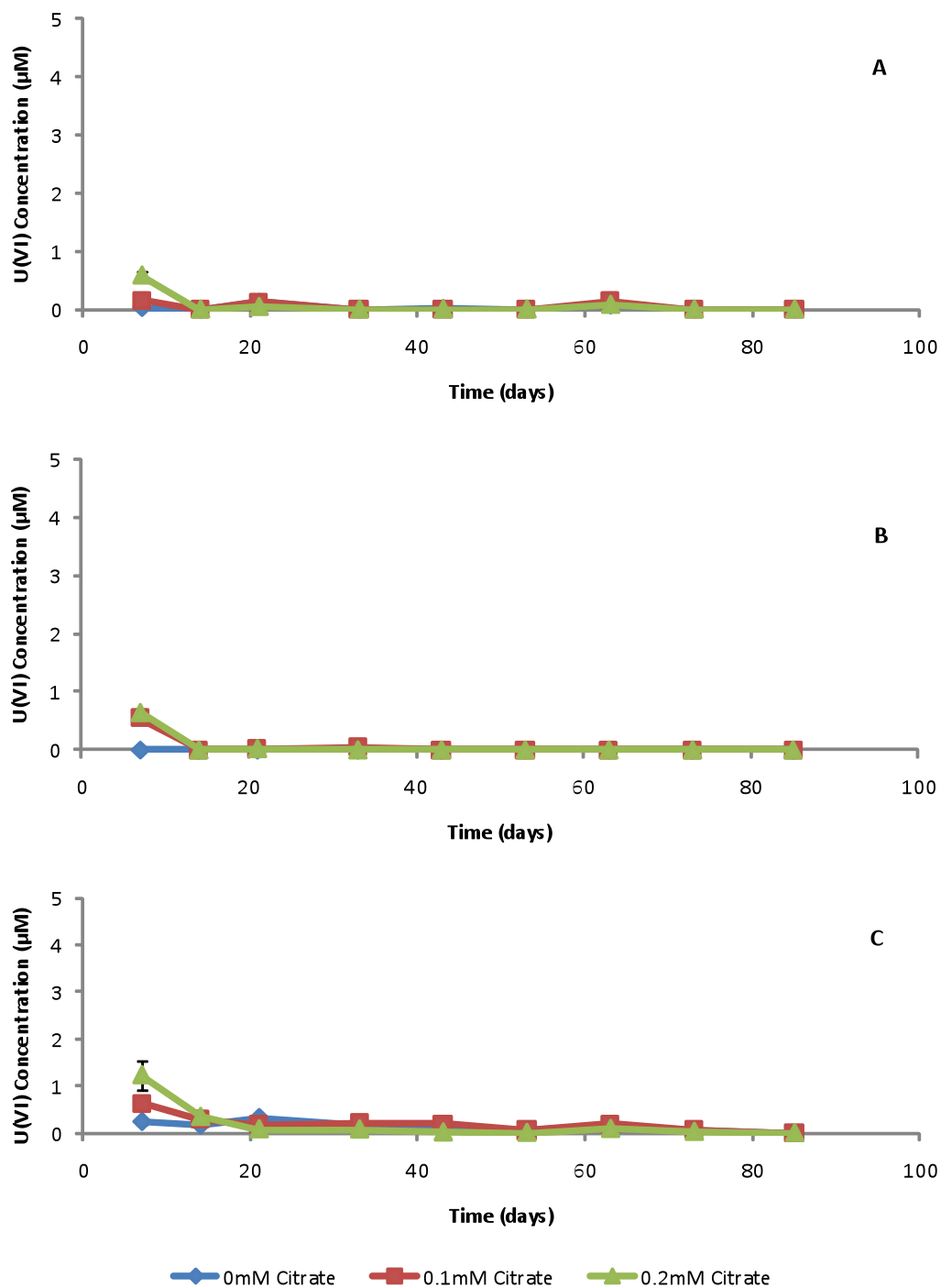


Figure 3.4. Dissolution of biogenic UO_2 by citrate showing U(VI) concentrations in 0mM bicarbonate (A), 3mM bicarbonate (B), and 10mM bicarbonate (C) showing citrate does not promote UO_2 dissolution or extraction of adsorbed U(VI). Error bars represent the standard deviation of the triplicates.

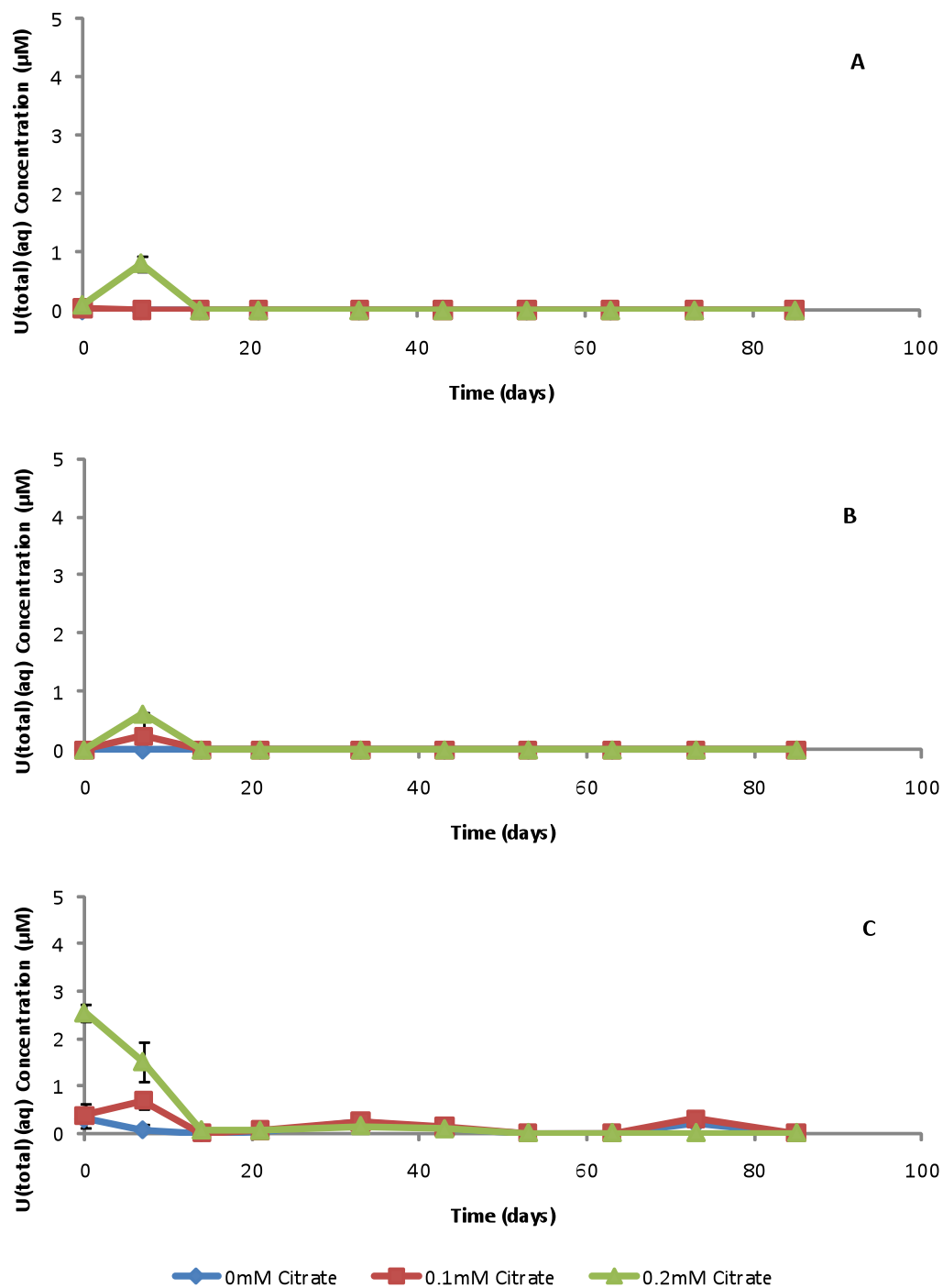


Figure 3.5. Dissolution of biogenic UO_2 by citrate in 0mM bicarbonate (A), 3mM bicarbonate (B), and 10mM bicarbonate (C) showing total aqueous U concentrations. Citrate does not promote UO_2 dissolution. Error bars represent the standard deviation of the triplicates.

Dissolution Experiments with EDTA and NTA

Figures 3.6 and 3.8 show total aqueous iron concentrations for EDTA and NTA promoted Fe(III) (hydr)oxide dissolution as a function of time with ferrihydrite, goethite, or hematite at pH 7 with 0mM and 10mM (Figure 3.7 and 3.9) bicarbonate. In all the EDTA systems, EDTA promoted dissolution compared to the EDTA-free control and 0.2mM EDTA promoted a greater extent of dissolution compared to 0.1mM EDTA with ferrihydrite and hematite. With goethite, the extent of aqueous iron dissolution was the same regardless of the concentration of EDTA. There was virtually no differences with the addition of 10mM bicarbonate to the rate and extent of Fe(III) (hydr)oxide promoted dissolution with EDTA and NTA. As seen in the previous experiments with DFB (Figure 3.1) and citrate (Figure 3.3), ferrihydrite was the most reactive followed by hematite, then goethite in the EDTA systems. DFB solubilized ferrihydrite at a non-zero-order $k_I = 0.037 \text{ day}^{-1}$, and the rate of ferrihydrite solubilization was greater with EDTA, $k_I = 0.043 \text{ day}^{-1}$ (Table 3.1). This was unexpected since DFB binds to Fe(III) stronger than EDTA binds to Fe(III); however, these experiments were only run once and new ferrihydrite was made before the start of every experiment potentially causing these unexpected results.

NTA promoted dissolution with ferrihydrite (Figures 3.8 and 3.9); however, very little dissolved iron was detected in the systems with goethite or hematite. When compared to EDTA (Table 3.1), NTA was not as efficient in the solubilization of ferrihydrite ($k_I = 0.0043$ and 0.043 day^{-1} for NTA and EDTA, respectively), which was expected since NTA binds to iron with a lower stability constant than EDTA. The rate of

solubilization of ferrihydrite was non-zero-order due to the extent of dissolution being greater with increasing NTA.

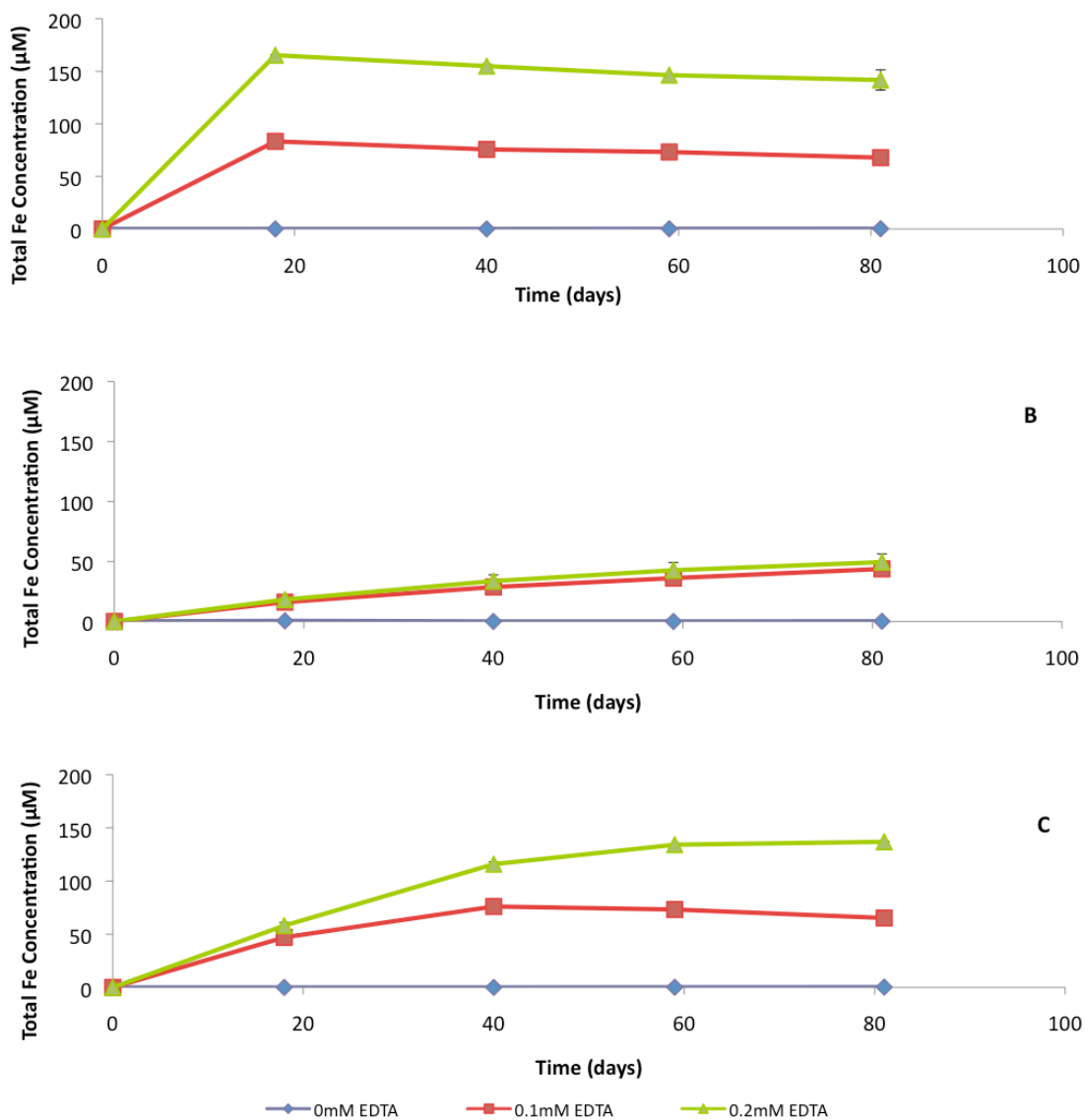


Figure 3.6. Dissolution of ferrihydrite (A), goethite (B), and hematite (C) by EDTA in 0mM bicarbonate showing EDTA promotes dissolution. Error bars represent the standard deviation of the triplicates.

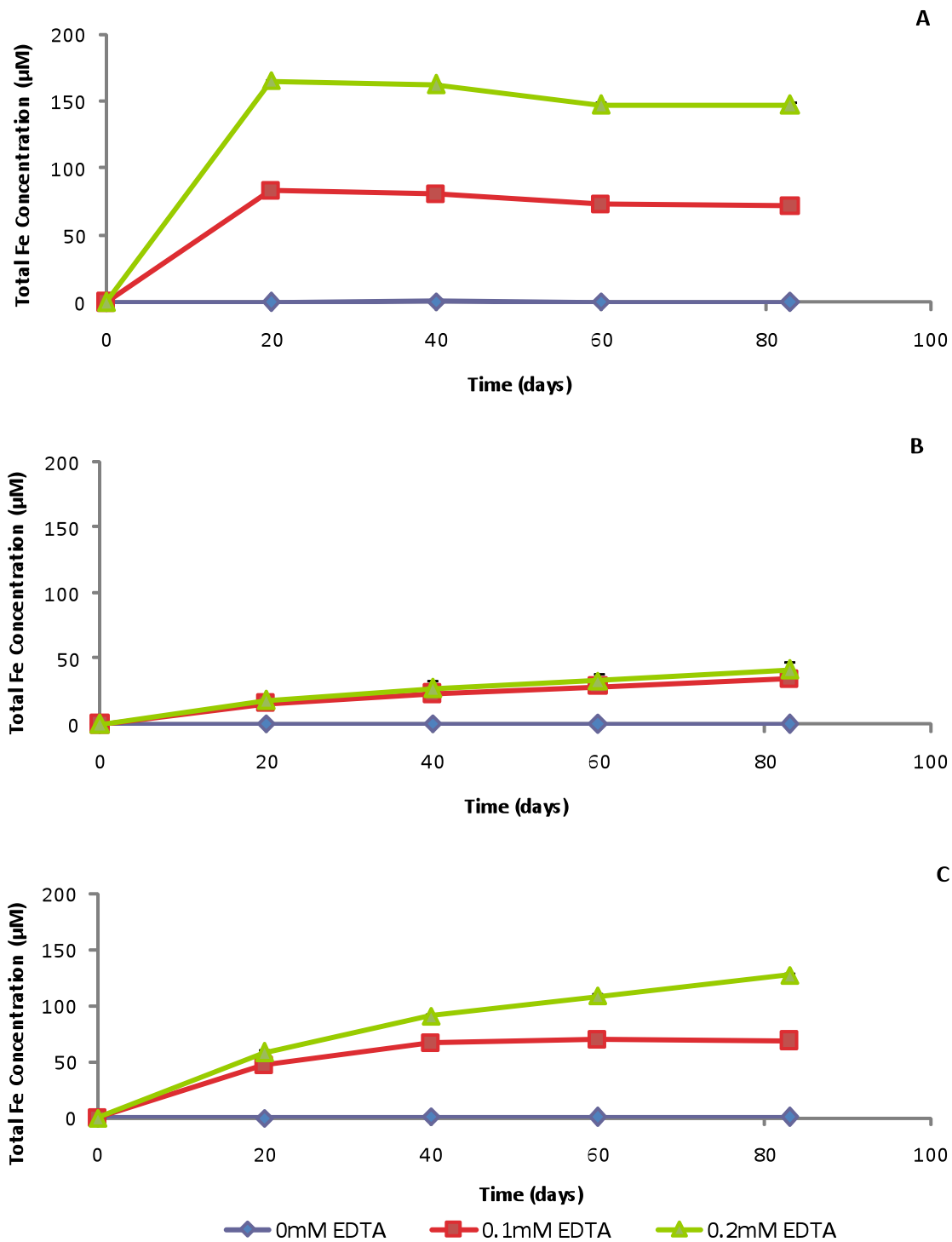


Figure 3.7. Dissolution of ferrihydrite (A), goethite (B), and hematite (C) by EDTA in 10mM bicarbonate showing EDTA promotes dissolution. Error bars represent the standard deviation of the triplicates.

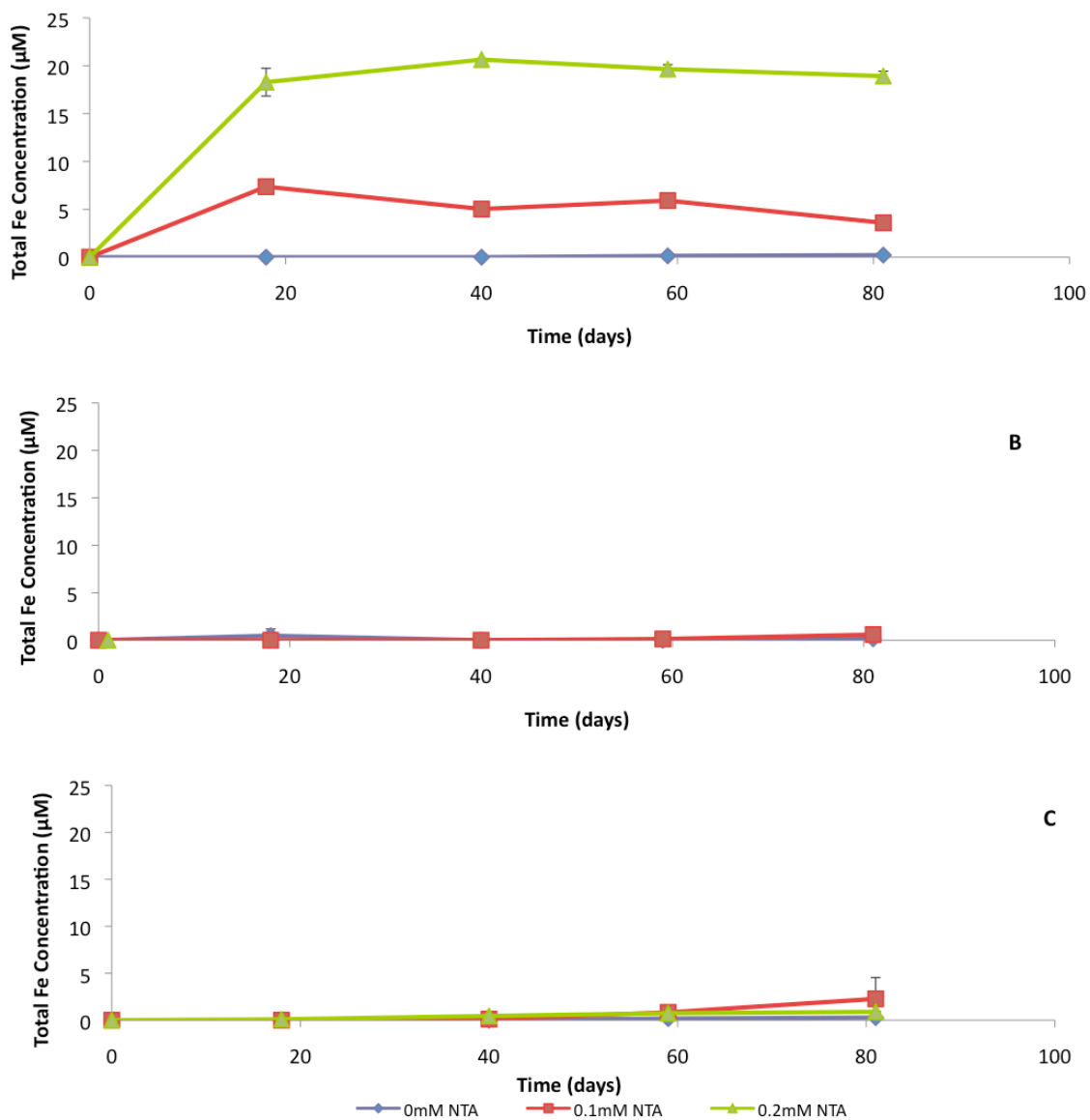


Figure 3.8. Dissolution of ferrihydrite (A), goethite (B), and hematite (C) by NTA in 0mM bicarbonate showing NTA promotes dissolution only with ferrihydrite. Error bars represent the standard deviation of the triplicates.

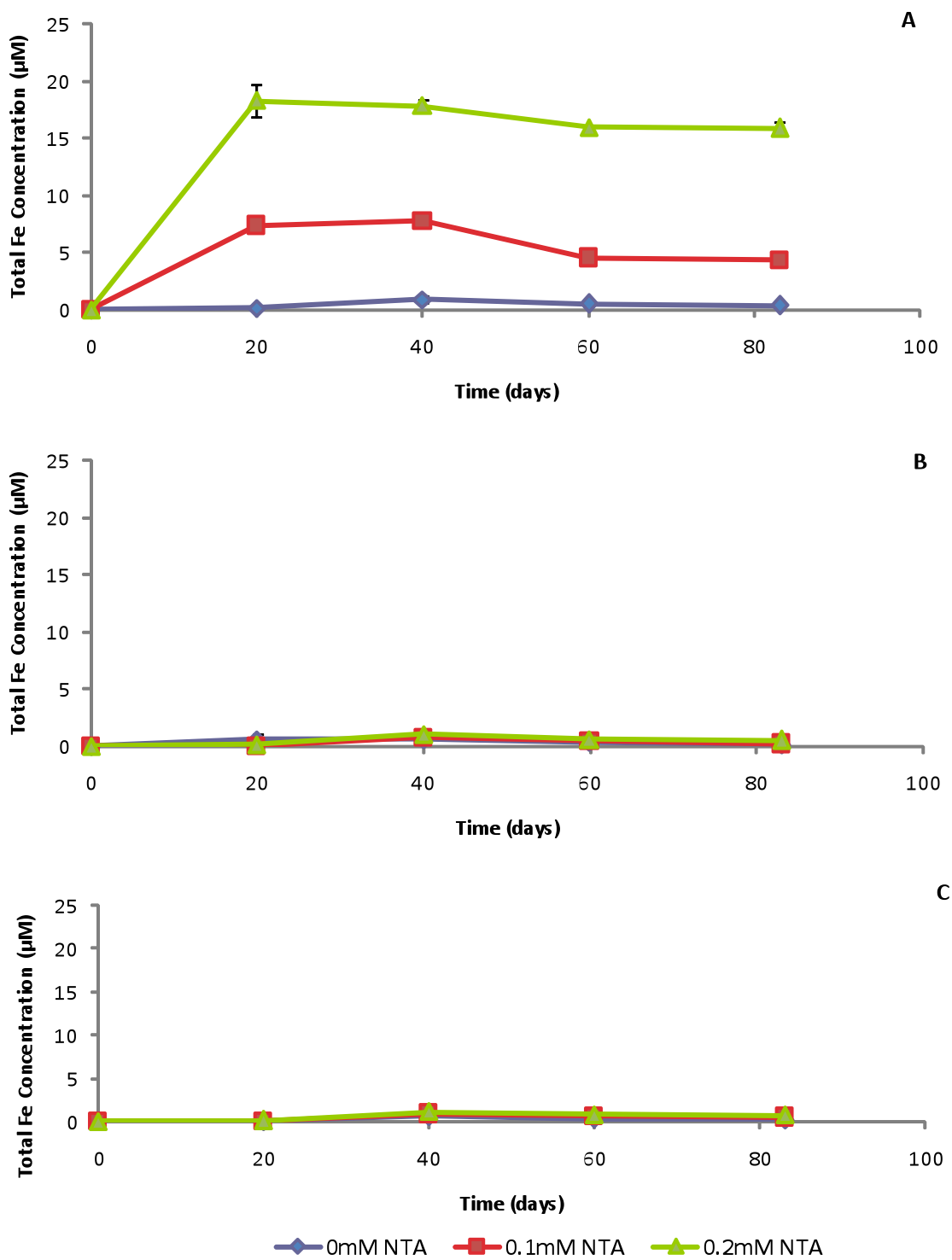


Figure 3.9. Dissolution of ferrihydrite (A), goethite (B), and hematite (C) by NTA in 10mM bicarbonate showing NTA promotes dissolution only with ferrihydrite. Error bars represent the standard deviation of the triplicates.

Figure 3.10 shows the changes in U(VI) concentrations and total dissolved U for EDTA or NTA at pH 7 with 10mM bicarbonate. In the systems with EDTA, U(VI) concentrations increased with increasing concentrations of EDTA showing EDTA likely extracted sorbed U(VI). Since the total dissolved U concentrations with EDTA are higher (at 10 days, total U was approximately $3\mu\text{M}$) than the U(VI) values (at 10 days, U(VI) was $< 1\mu\text{M}$), it appears that EDTA promotes UO_2 dissolution by increasing

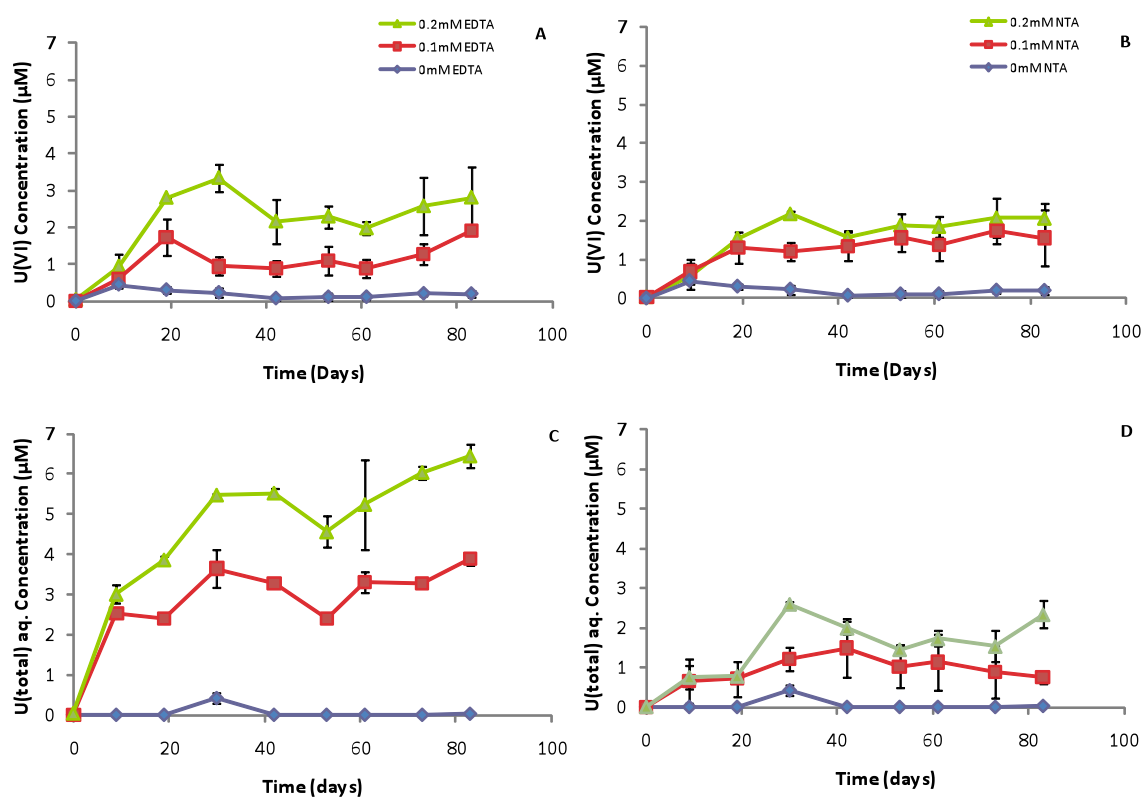


Figure 3.10. Dissolution of biogenic UO_2 by EDTA (A, C) and NTA (B, D) in 10mM bicarbonate showing U(VI) concentrations (A, B) and total dissolved U (C, D) showing EDTA promotes UO_2 dissolution while NTA affected dissolution through U(VI) extraction. Error bars represent the standard deviation of the triplicates.

aqueous U(IV). This reaction had a non-zero-order $k_1 = 0.0009 \text{ day}^{-1}$ (Table 3.2) for

U(VI). In the systems with NTA, all U(VI) and total dissolved U values were virtually

the same and below 3 μ M suggesting NTA does not promote UO₂ dissolution. However, comparing U(VI) values in the systems with NTA to the NTA-free controls, NTA did increase extraction of U(VI) that was adsorbed onto the UO₂ particles.

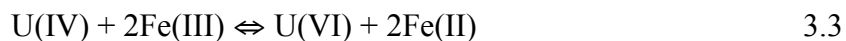
In contrast to DFB, citrate, and NTA, EDTA was the only chelator tested that promoted UO₂ dissolution. DFB and NTA only increased the amount of aqueous U(VI) through extraction of U(VI) adsorbed to UO₂ particles. Citrate did not promote UO₂ dissolution nor was it able to extract adsorbed U(VI).

UO₂ Reoxidation Experiments

The chelators, DFB, citrate, NTA, and EDTA were investigated for their ability to reoxidize UO₂ in the presence and absence of Fe(III) (hydr)oxides and their rates were quantified. Also, UO₂ reoxidation was investigated by aqueous Fe(III), which has previously been shown to rapidly reoxidize UO₂. Fe(III) bound to a chelator such as DFB or citrate was also tested for its ability to enhance UO₂ reoxidation rates.

Reoxidation of U by Ferric Chloride

Figure 3.11 shows the time course of dissolved U(VI) with FeCl₃-free control (pH 2.3), 1.25mM FeCl₃ (pH 3.1), 2.5mM FeCl₃ (pH 2.7), and 25mM FeCl₃ (pH 2.2). Iron added to the anoxic system as aqueous FeCl₃ in excess or in equal amounts (stoichiometrically), resulted in oxidation of UO₂ within one hour. Two moles of Fe(III) are needed to completely reoxidize one mole of U(IV) as shown in Figure 3.11, according to Equation 3.3:



In this system, the pH was between 2 and 3 and could not be adjusted higher due to the formation of solid iron minerals. At higher pH values, we expect the rate of reoxidation to be slower due to the formation of Fe(III) (hydr)oxides.

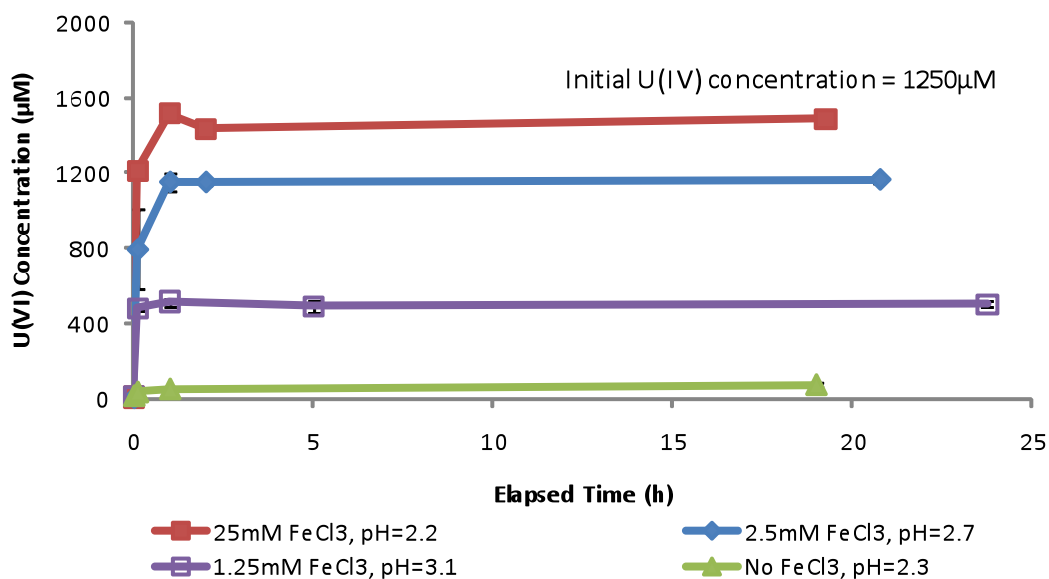


Figure 3.11. Reoxidation of UO_2 by aqueous Fe(III) added as FeCl_3 at different pH values showing Fe(III) completely reoxidizes U. Error bars represent the standard deviation between triplicates.

Reoxidation of U by DFB Bound to Fe(III)

Dissolved U concentrations are shown in Figure 3.12 with 1 mol DFB: 1 mol Fe(III) complex added as FeCl_3 bound to DFB at pH 2.1, pH 5, pH 7.1. Figure 3.12 also shows dissolved U concentrations for the complex, 2 mol Fe(III): 1 mol DFB at pH 2.5. When Fe(III) was added to UO_2 in the form of Fe(III) bound 1:1 with DFB, no reoxidation was observed at pH 5 or 7. At pH 2, some reoxidation was observed; however, when compared to the DFB-free control, these values were similar and reoxidation of UO_2 is likely due to the excess H^+ ions rather than DFB (Figure 3.11).

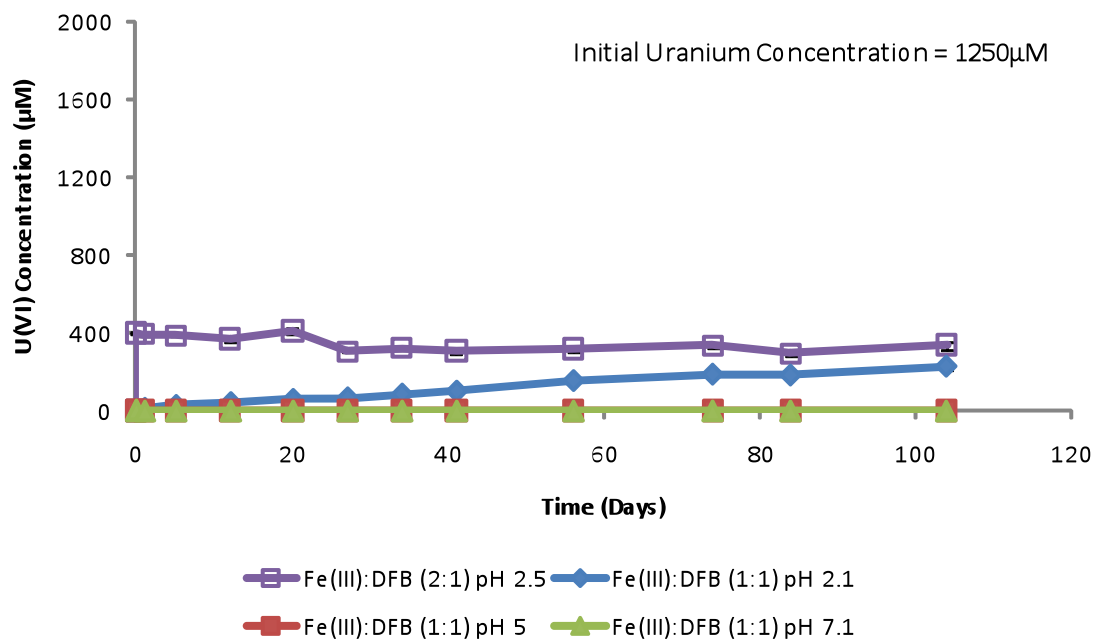


Figure 3.12. Reoxidation of UO_2 by 1.25mM Fe(III) bound to 1.25mM DFB at pH 2.1, 5, 7.1 and 2.5mM Fe(III) bound to 1.25mM DFB. Some reoxidation was seen with the 2:1 complex of Fe(III):DFB at pH 2.1 while no reoxidation was seen at the higher pH values. Error bars represent the standard deviation of the triplicates.

A mixture of 2.5mM of Fe(III) bound to 1.25mM DFB only oxidized approximately a third of the total U available (roughly 400µM U(VI) was detected) as shown in Figure 3.12. These results indicate that when Fe(III) is bound to DFB, iron is not reactive with UO_2 . With a high stability constant ($\log K = 30.6$) one possible explanation is that DFB binds to Fe(III) so strongly that there is virtually no free Fe(III) available to reoxidize UO_2 . Some reoxidation is observed when not all Fe(III) is bound to DFB (open squares in Figure 3.12). Thus, adding DFB to the system inhibits UO_2 reoxidation by Fe(III).

Reoxidation of U by Ferric Citrate

U(VI) concentrations for 2.5mM ferric citrate at pH 2.6, pH 5.4, and pH 7.1 are shown in Figure 3.13. When comparing ferric citrate with the results from FeDFB treatments (Figure 3.10) at pH 2, ferric citrate reoxidized more UO_2 than FeDFB, but again no reoxidation was observed at higher pH values. Ferric citrate only reoxidized approximately half of the total U available (approximately $600\mu\text{M}$ UO_2 was reoxidized) in two days whereas with FeDFB less reoxidation was observed ($< 30\mu\text{M}$ UO_2 was reoxidized in that time). Citrate chelates Fe(III) with a lower stability constant than DFB.

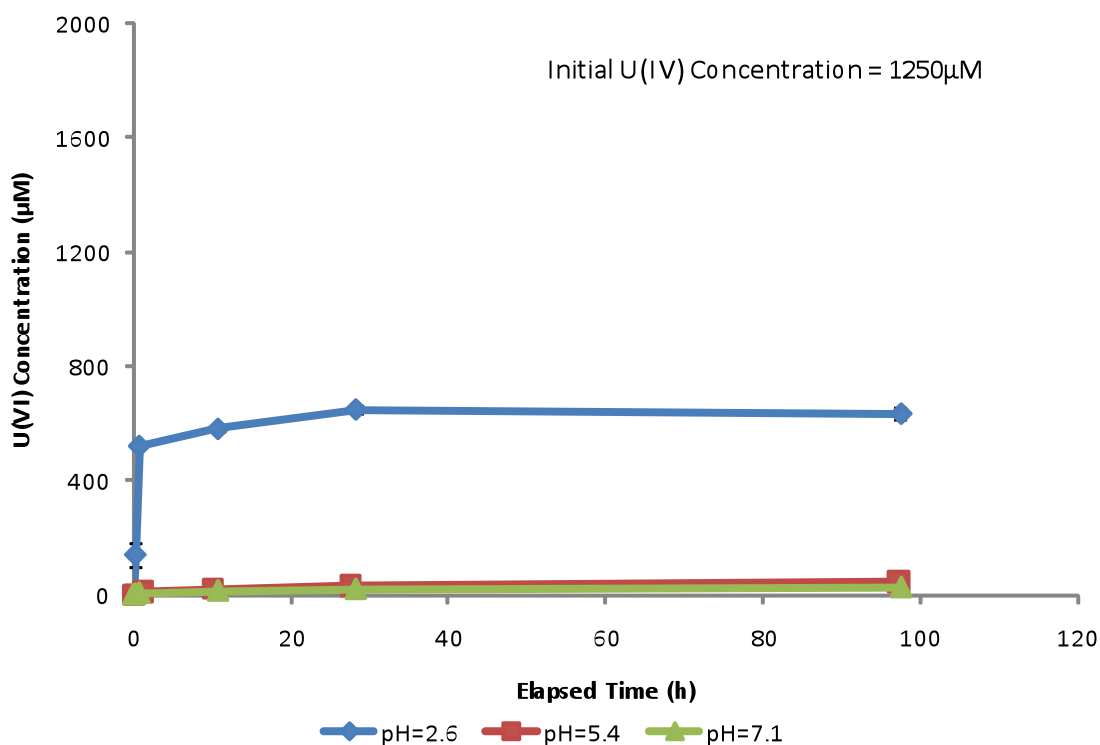


Figure 3.13. Reoxidation of UO_2 by 2.5mM ferric citrate at different pH values showing ferric citrate can reoxidize UO_2 at low pH but not at higher pH values. Error bars represent the standard deviation of the triplicates.

This might allow for more free aqueous Fe(III) to be available to reoxidize UO_2 compared to DFB, which could explain the difference in U(VI) values. The amount of reoxidation of UO_2 observed is also dependent on pH, where at higher pH values, less reoxidation occurs.

These results indicate that chelators found in nature such as citrate and DFB, may be important in determining U mobility as well as affecting the overall stability of U. Excess dissolved Fe(III) at $\text{pH} < 3$, reoxidized UO_2 completely in approximately one hour; however, when Fe(III) was chelated, Figure 3.12 shows some was observed in systems with DFB and only partial reoxidation was observed with citrate (Figure 3.13). One possible explanation could be due to the difference in stability constants between the chelator and Fe(III). As chelators are introduced into environments with excess aqueous Fe(III), the amount of free Fe(III) becomes less, resulting in less overall UO_2 reoxidation. The high stability constant with DFB and Fe(III) results in less free Fe(III) available to reoxidize UO_2 since most Fe(III) would be found in its chelated form and thus, no reoxidation of UO_2 was observed. However, with a much lower stability constant between citrate and Fe(III), partial reoxidation of UO_2 was observed as a result of greater amounts of aqueous Fe(III) being available to partake in reoxidation.

Reoxidation of U in the Presence of DFB and Fe(III) (hydr)oxides

U Reoxidation in the Presence of DFB and Ferrihydrite

Dissolved U(VI) values with DFB and ferrihydrite present at different bicarbonate concentrations at pH 7 are shown in Figure 3.14. With DFB, there was less reoxidation of UO_2 in the presence of ferrihydrite and bicarbonate (0mM, 3mM, and 10mM) when

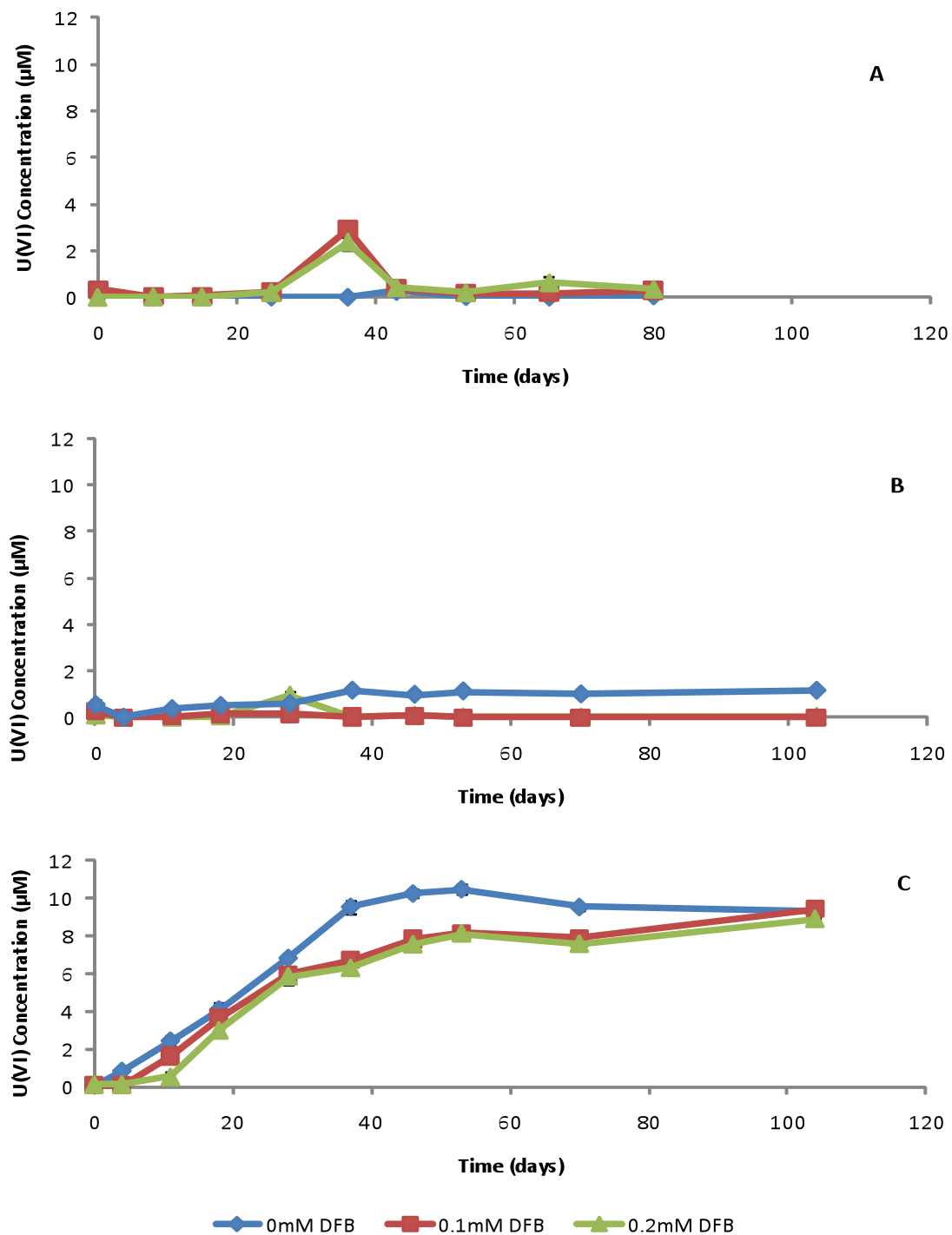


Figure 3.14. UO_2 reoxidation in the presence of DFB and ferrihydrite with 0mM (A), 3mM (B), and 10mM (C) bicarbonate added showing DFB inhibits UO_2 reoxidation. Error bars represent the standard deviation of the triplicates.

compared to the DFB-free controls. In systems with 0mM or 3mM bicarbonate, all U(VI) concentrations were below 3 μ M and no reoxidation was observed. However, when 10mM bicarbonate was added, about 10 μ M of UO₂ was reoxidized to U(VI) in the systems with no DFB. In Fe(III)-free experiments with 10mM bicarbonate (Figure 3.2), U(VI) concentrations remained low (< 2 μ M) indicating ferrihydrite could reoxidize UO₂ only at 10mM bicarbonate concentration without the help of a chelator. These results are consistent with the results from Ginder-Vogel et al. (2006) where they demonstrated that UO₂ reoxidation by ferrihydrite is favorable in the presence of high bicarbonate concentrations. Adding DFB to the systems did not increase U(VI) concentrations, nor the initial rates of reoxidation (Table 3.3). Virtually no aqueous U(VI) was detected in the systems with no bicarbonate. In the systems with 3mM and 10mM bicarbonate (Figure 3.12C), adding DFB decreased the rate of UO₂ reoxidation by ferrihydrite.

One possible explanation is that DFB could be binding to the surface of ferrihydrite making it less reactive with UO₂. Another possibility is that DFB initially chelates all the dissolved Fe(III), delaying UO₂ reoxidation until sufficient amounts of dissolved Fe(III) are available to react with the UO₂. In dissolution experiments with DFB and UO₂, DFB was only able to extract U(VI) adsorbed to solid surfaces. In systems with DFB and ferrihydrite only, DFB was shown to promote ferrihydrite dissolution, but DFB bound to Fe(III) was unable to reoxidize UO₂ (also verified in Figure 3.12). Since DFB has a higher affinity for chelating Fe(III) than U(VI), then another possible mechanism is DFB only binding to Fe(III) on ferrihydrite, promoting dissolution of ferrihydrite. However, once DFB binds to Fe(III), DFB does not dissociate

Table 3.3. Table of rate constant results from UO_2 reoxidation experiments with ferrihydrite (Ferri), goethite (Goe), and hematite (Hem) with DFB, citrate, EDTA, and NTA with bicarbonate (0mM, 3mM, or 10mM).

	U(VI)				
	Zero-Order k [$\mu\text{mol U(VI)}/\text{L}^* \text{ day}$]			Non-zero-Order k [1/day]	
UO_2 Reoxidation	Standard Deviation			Standard Deviation	
DFB + Ferri + 0Bi	0		0	0	0
DFB + Ferri + 3Bi	0		0	0	0
DFB + Ferri + 10Bi	2.1E-01	±	3.5E-02	--	--
DFB + Goe + 0Bi	0		0	0	0
DFB + Goe + 3Bi	0		0	0	0
DFB + Goe + 10Bi	0		0	0	0
DFB + Hem + 0Bi	0		0	0	0
DFB + Hem + 3Bi	0		0	0	0
DFB + Hem + 10Bi	0		0	0	0
Cit + Ferri + 0Bi	0		--	0	0
Cit + Ferri + 3Bi	1.0E-02	±	1.0E-02	--	--
Cit + Ferri + 10Bi	--		--	9.0E-03	± 4.7E-04
Cit + Goe + 0Bi	0		0	0	0
Cit + Goe + 3Bi	0		0	0	0
Cit + Goe + 10Bi	5.2E-02	±	1.7E-02	--	--
Cit + Hem + 0Bi	0		0	0	0
Cit + Hem + 3Bi	0		0	0	0
Cit + Hem + 10Bi	6.9E-02	±	1.5E-02	--	--
EDTA + Ferri + 10Bi	--		--	4.9E-02	± 2.9E-03
EDTA + Goe + 10Bi	--		--	4.4E-02	± 2.9E-03
EDTA + Hem + 10Bi	--		--	4.6E-02	± 6.1E-03
NTA + Ferri + 10Bi	--		--	2.6E-02	± 2.1E-03
NTA + Goe + 10Bi	--		--	3.8E-03	± 5.8E-04
NTA + Hem + 10Bi	--		--	4.0E-03	± 8.1E-04
Ferri + 10Bi	2.0E-01	±	8.4E-02	--	--
Goe + 10Bi	5.8E-02	±	3.9E-02	--	--
Hem + 10Bi	1.4E-02	±	4.1E-03	--	--

0Bi, 3Bi, and 10Bi are 0mM, 3mM, and 10mM bicarbonate concentrations

from Fe(III) leaving UO_2 in an environment surrounded by aqueous Fe(III), but unavailable for electron transfer due to being chelated with DFB.

U Reoxidation in the Presence of DFB and Goethite or Hematite

Figure 3.15 displays the dissolved U(VI) concentrations with DFB and goethite and Figure 3.16 with DFB and hematite at various bicarbonate concentrations at pH 7. With goethite and hematite, adding DFB increased U(VI) concentrations compared to the DFB-free controls. However, after approximately 100 days the amount of U(VI) was $< 15\mu\text{M}$ with 0.2mM DFB and $< 10\mu\text{M}$ with 0.1mM DFB and virtually the same as the Fe(III)-free controls (open symbols in Figure 3.15 and 3.16). This suggests DFB does not promote UO_2 oxidation in the presence of goethite or hematite. With ferrihydrite in the DFB-free controls (Figure 3.14), U(VI) values were higher (roughly $10\mu\text{M}$) with 10mM bicarbonate versus goethite or hematite where U(VI) concentrations were $< 2\mu\text{M}$ implying ferrihydrite reoxidized UO_2 under these conditions while goethite and hematite have little to no ability to reoxidize UO_2 . With ferrihydrite and 0mM or 3mM bicarbonate, U(VI) concentrations were $< 2\mu\text{M}$ and ferrihydrite promoted slight reoxidation. With hematite and goethite, U(VI) was not detected at these conditions suggesting goethite and hematite were unable to reoxidize UO_2 at all conditions tested. Thus, any soluble U(VI) values were the result of DFB and/or bicarbonate solubilizing the U(VI) adsorbed onto the UO_2 particles in both the systems with goethite or hematite since adding the Fe(III) (hydr)oxide to the systems did not affect the results as seen with the Fe(III)-free controls (open symbols in Figure 3.15 and 3.16).

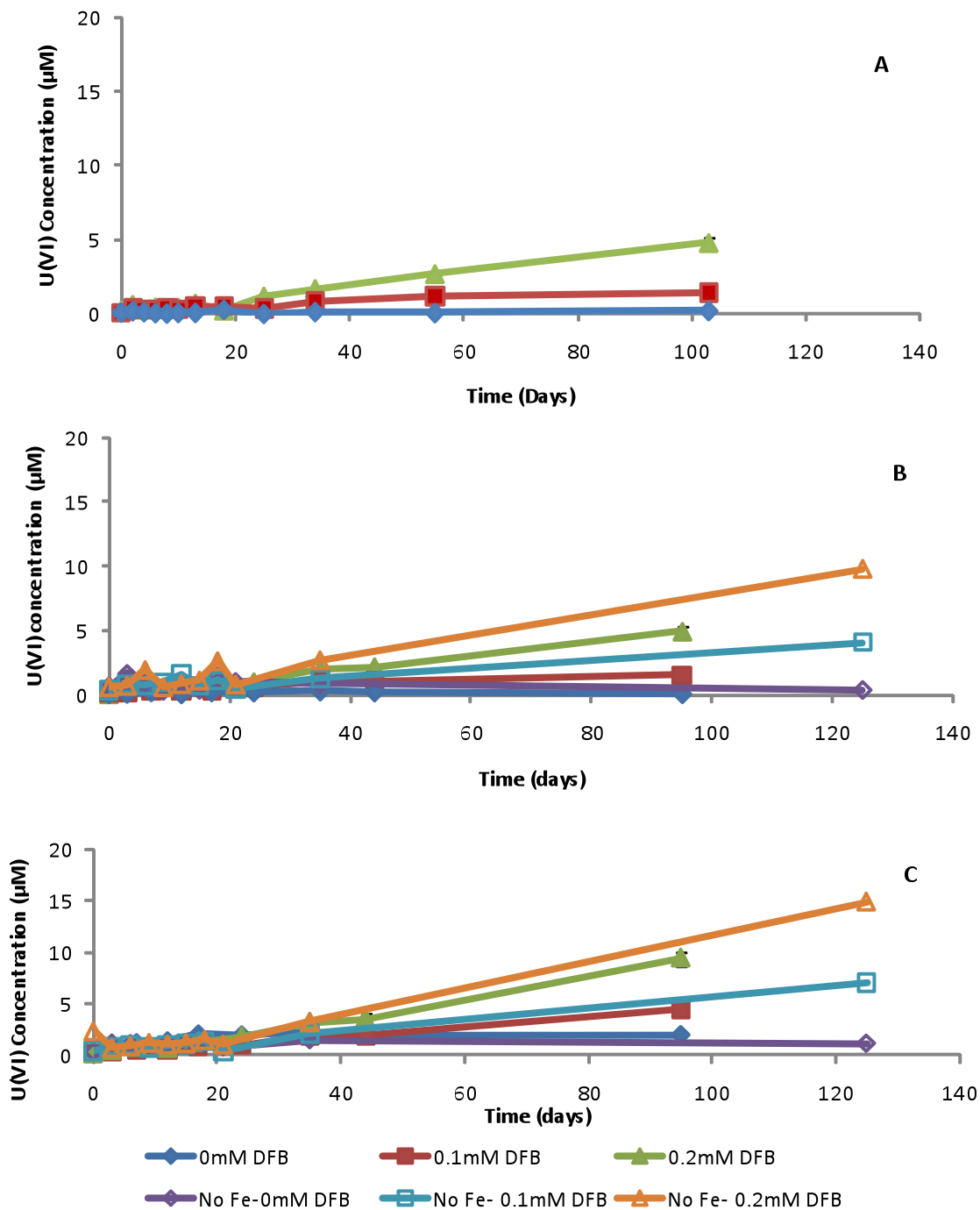


Figure 3.15. UO_2 reoxidation in the presence of DFB and goethite in 0mM bicarbonate (A), 3mM bicarbonate (B), and 10mM bicarbonate (C) with Fe-free controls showing goethite and DFB do not promote UO_2 reoxidation. Error bars represent the standard deviation of the triplicates.

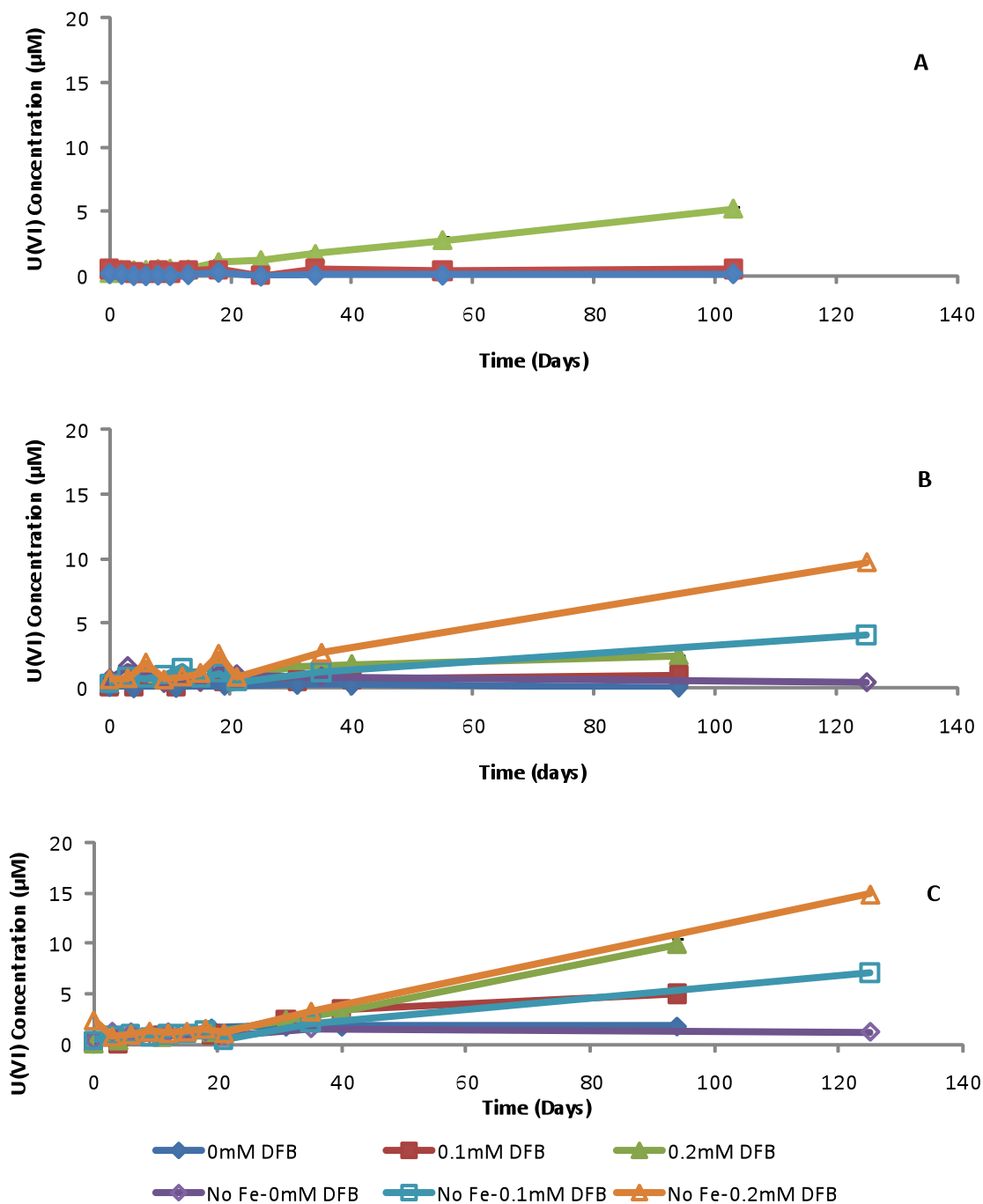


Figure 3.16. UO₂ reoxidation in the presence of DFB and hematite with 0mM bicarbonate (A), 3mM bicarbonate (B), and 10mM bicarbonate (C) with Fe-free controls showing hematite and DFB do not promote UO₂ reoxidation. Error bars represent the standard deviation of the triplicates.

Reoxidation of U in the Presence of Citrate and Fe(III) (hydr)oxides

U Reoxidation in the Presence of Citrate and Ferrihydrite

U(VI) concentrations with citrate, ferrihydrite, and bicarbonate at pH 7 were measured over time (Figure 3.17). In the citrate-free control, UO_2 reoxidation by ferrihydrite was observed in only the 10mM bicarbonate systems and roughly 15 μM of U(VI) was detected, which is similar to the results seen in the DFB-free control with ferrihydrite. In the citrate-free control with 0mM bicarbonate, ferrihydrite was unable to reoxidize UO_2 , and with 3mM bicarbonate, very little UO_2 was reoxidized by ferrihydrite ($< 3\mu\text{M}$). The rate of reoxidation of UO_2 by ferrihydrite in the presence of citrate was a non-zero-order reaction with an initial rate constant of $k_I = 0.009 \text{ day}^{-1}$ (Table 3.3) in systems with 10mM bicarbonate, but leveled off after approximately 20 days. Since citrate did not promote dissolution of UO_2 (Figure 3.4 and 3.5), the rate accelerating step appears to be citrate promoting dissolution of ferrihydrite, which allows the soluble Fe(III) to react with UO_2 . In the systems with 0mM or 3mM bicarbonate, there was virtually no U(VI) present, which was similar to the systems with ferrihydrite and DFB. Only at 10mM bicarbonate concentrations did U reoxidation occur, and citrate increased the rate of UO_2 reoxidation dependent on the amount of citrate present, but not the extent.

Since citrate increased the rate of reoxidation by ferrihydrite and DFB inhibited reoxidation, one explanation is based on the difference in their stability constants with Fe(III). Citrate binds to Fe(III) less strongly than DFB enabling more free aqueous Fe(III) to react with UO_2 whereas Fe(III) binds very strongly to DFB, making Fe(III) virtually unavailable to reoxidize biogenic UO_2 .

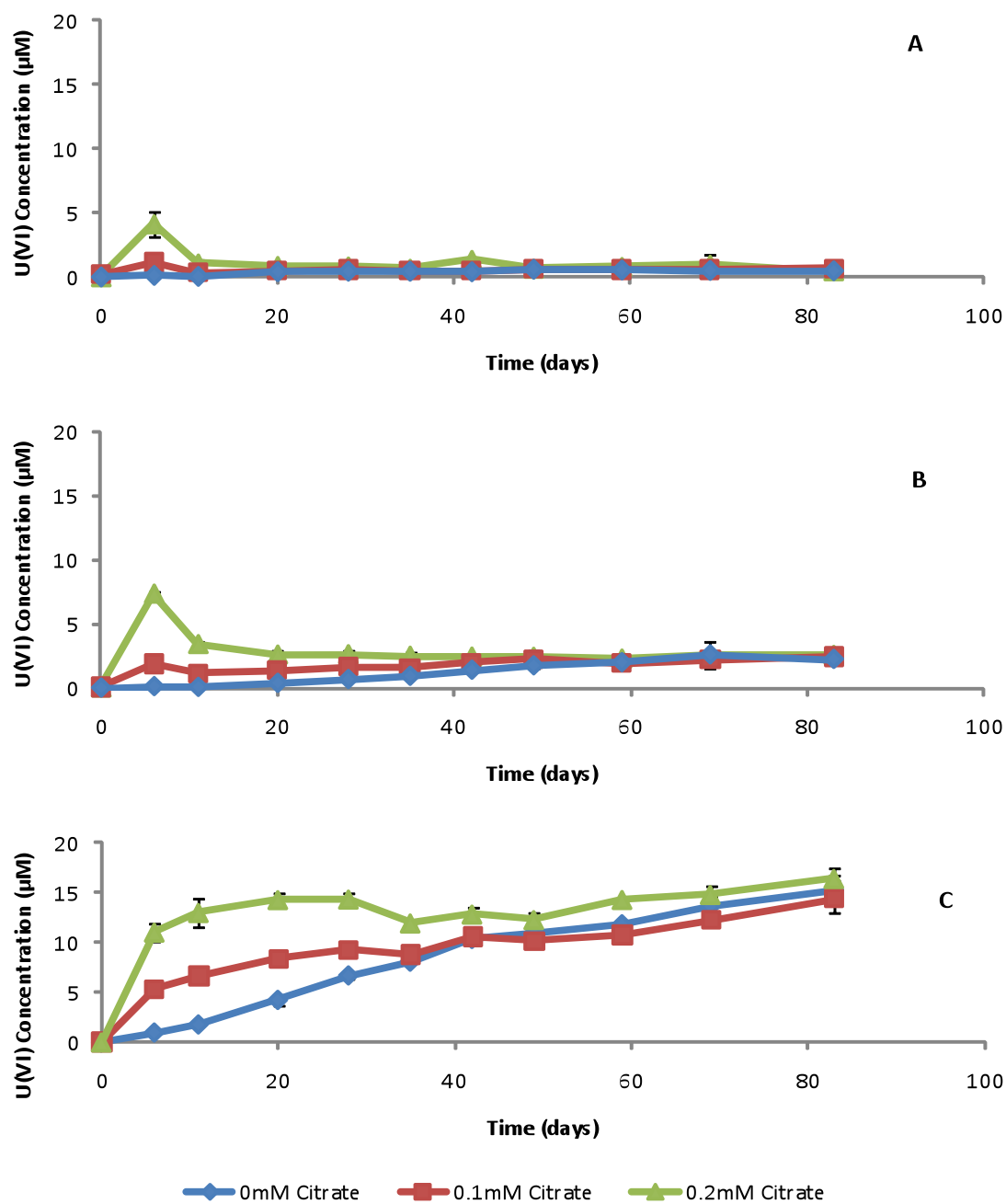


Figure 3.17. UO_2 reoxidation in the presence of citrate and ferrihydrite with 0mM bicarbonate (A), 3mM bicarbonate (B), and 10mM bicarbonate (C) showing citrate increases the rate of U reoxidation only with 10mM bicarbonate, but not the extent. Error bars represent the standard deviation of the triplicates.

U reoxidation in the presence of citrate and goethite or hematite

Dissolved U(VI) concentrations were tracked over 80 days with citrate in the presence of goethite or hematite at various bicarbonate concentrations at pH 7 (Figure 3.18 and 3.19). There was virtually no dissolved U(VI) detected with citrate and hematite or goethite at 0mM and 3mM bicarbonate concentrations. With 10mM bicarbonate, some reoxidation was observed with goethite and hematite in the citrate-free control; however, these values are $< 2\mu\text{M}$. It appears that 0.2mM citrate may have slightly inhibited UO_2 reoxidation when compared to the citrate-free controls; however, the difference in values is approximately $1\mu\text{M}$ so citrate inhibition of UO_2 reoxidation may not be significant. Compared to ferrihydrite, goethite and hematite are less reactive and do not play as significant a role in UO_2 reoxidation with citrate.

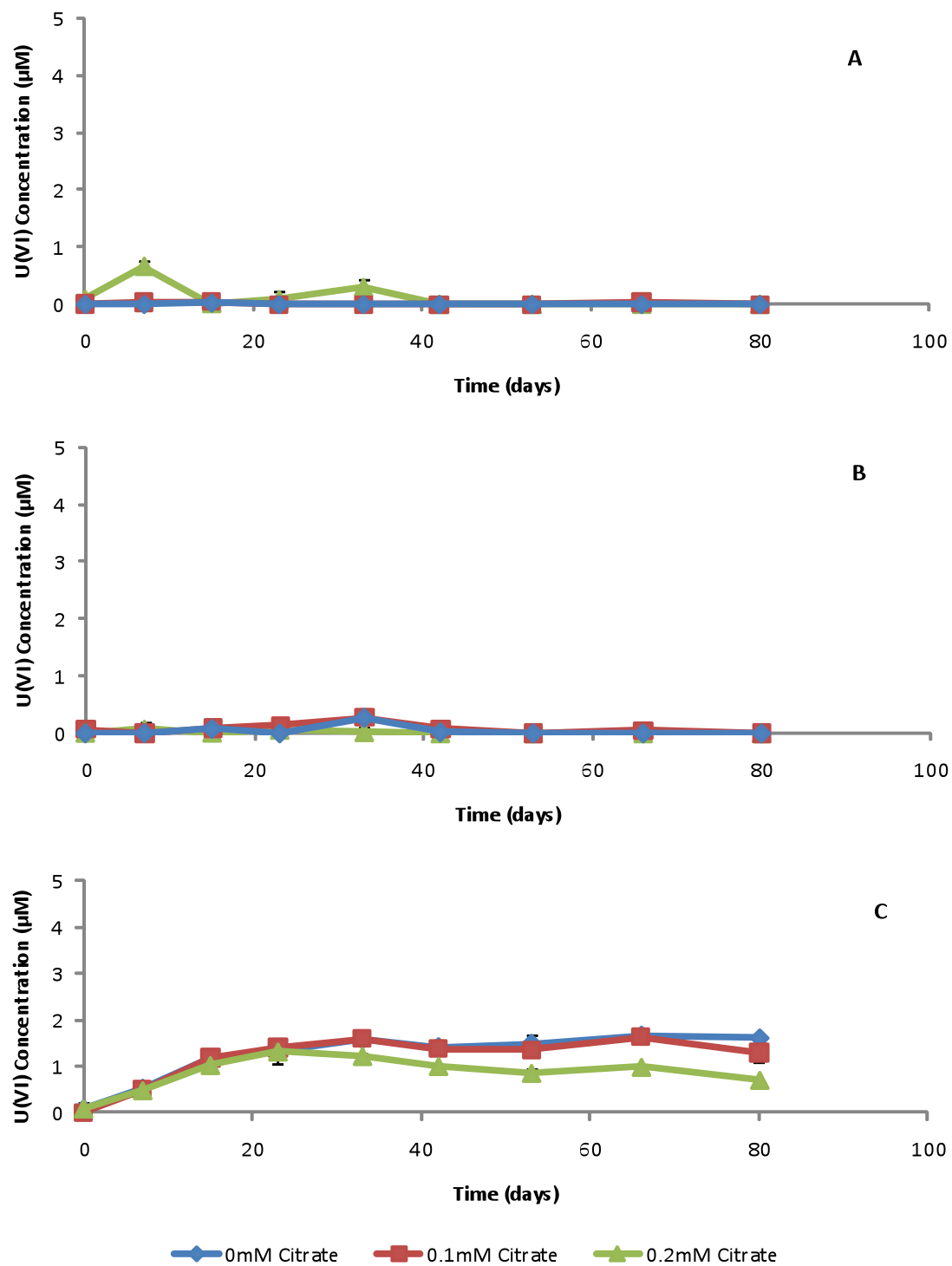


Figure 3.18. UO_2 reoxidation in the presence of citrate and goethite with 0mM bicarbonate (A), 3mM bicarbonate (B), and 10mM bicarbonate (C) showing citrate may slightly inhibit UO_2 reoxidation. Error bars represent the standard deviation between the triplicates.

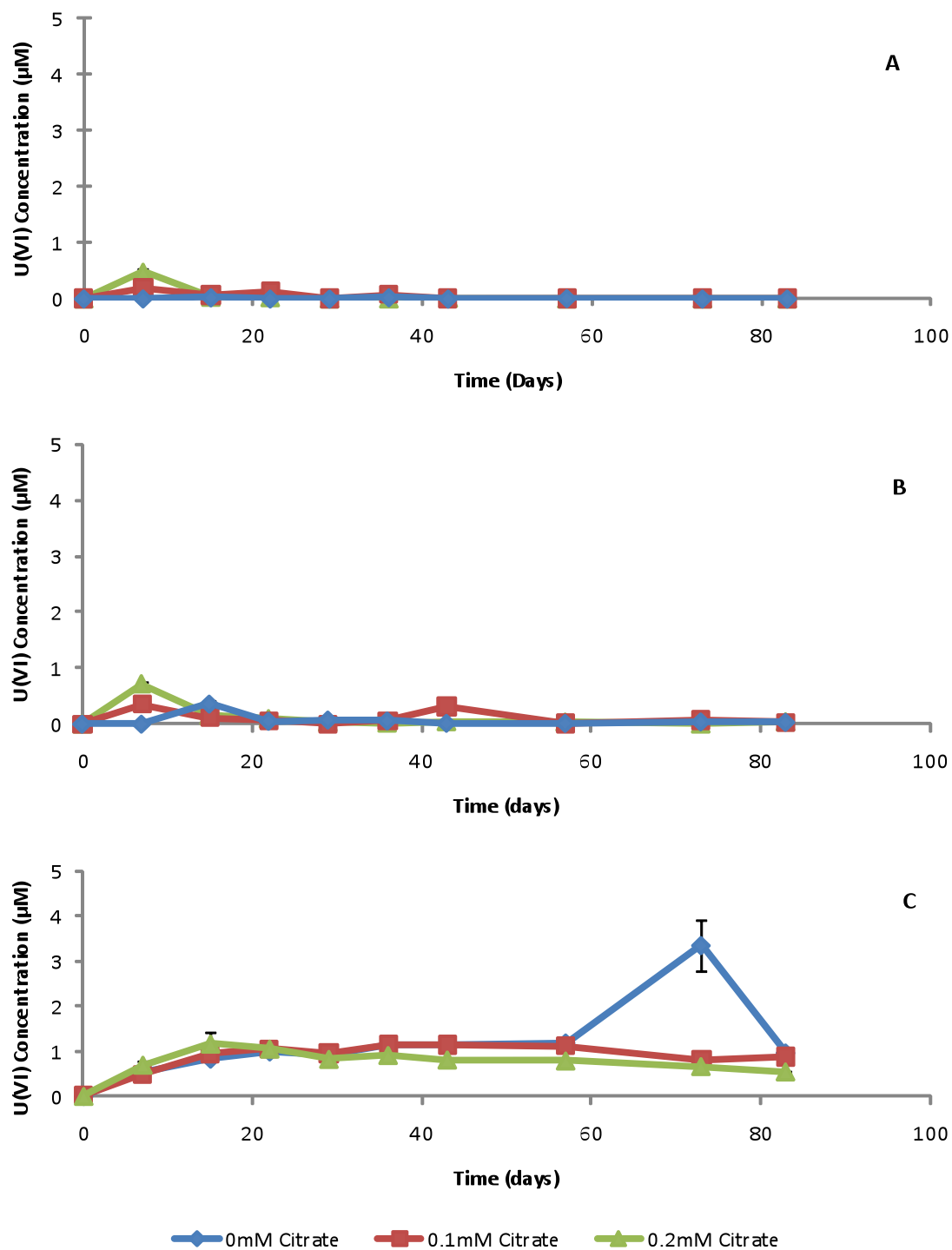


Figure 3.19. UO_2 reoxidation in the presence of citrate and hematite with 0mM bicarbonate (A), 3mM bicarbonate (B), or 10mM bicarbonate (C) showing citrate may inhibit UO_2 reoxidation. Error bars represent the standard deviation between the triplicates.

Reoxidation of U in the Presence of EDTA/NTA and Fe(III) (hydr)oxides

U Reoxidation in the Presence of EDTA and Fe(III) (hydr)oxides

Ferrihydrite, goethite, and hematite were tested for their ability to reoxidize UO_2 in the presence of EDTA. In Figure 3.20, it can be seen the rate and extent of UO_2 reoxidation by iron minerals with EDTA is significantly higher than observed in systems with either DFB or citrate. With EDTA within the first week, approximately 60 to 70 μM UO_2 was reoxidized (giving a non-zero-order rate constant $k_I = 0.049, 0.044,$ and 0.046 day^{-1} for ferrihydrite, goethite, and hematite, respectively) and was independent of the Fe(III) (hydr)oxide present. Compared to citrate ($k_I = 0.009 \text{ day}^{-1}$) (Table 3.3), EDTA reoxidized UO_2 at a greater rate independent of the Fe(III) (hydr)oxide present. The amount of U(VI) detected was dependent on the concentration of EDTA present. When comparing the three Fe(III) (hydr)oxides, U(VI) values were very similar indicating that EDTA pulling U into solution is an important mechanism in this case, in addition to potential solubilization of Fe(III) (hydr)oxides by EDTA. Since EDTA increased dissolution rates of UO_2 and Fe(III)-(hydr)oxides, UO_2 reoxidation was much more extensive than with citrate, where only iron was being solubilized. EDTA was the only Fe(III)-chelator tested capable of promoting UO_2 dissolution. EDTA also binds less strongly with Fe(III) compared to DFB, allowing for potentially more Fe(III) freely available to reoxidize UO_2 . EDTA promotes UO_2 reoxidation through solubilizing of UO_2 , independent of the Fe(III) (hydr)oxide present.

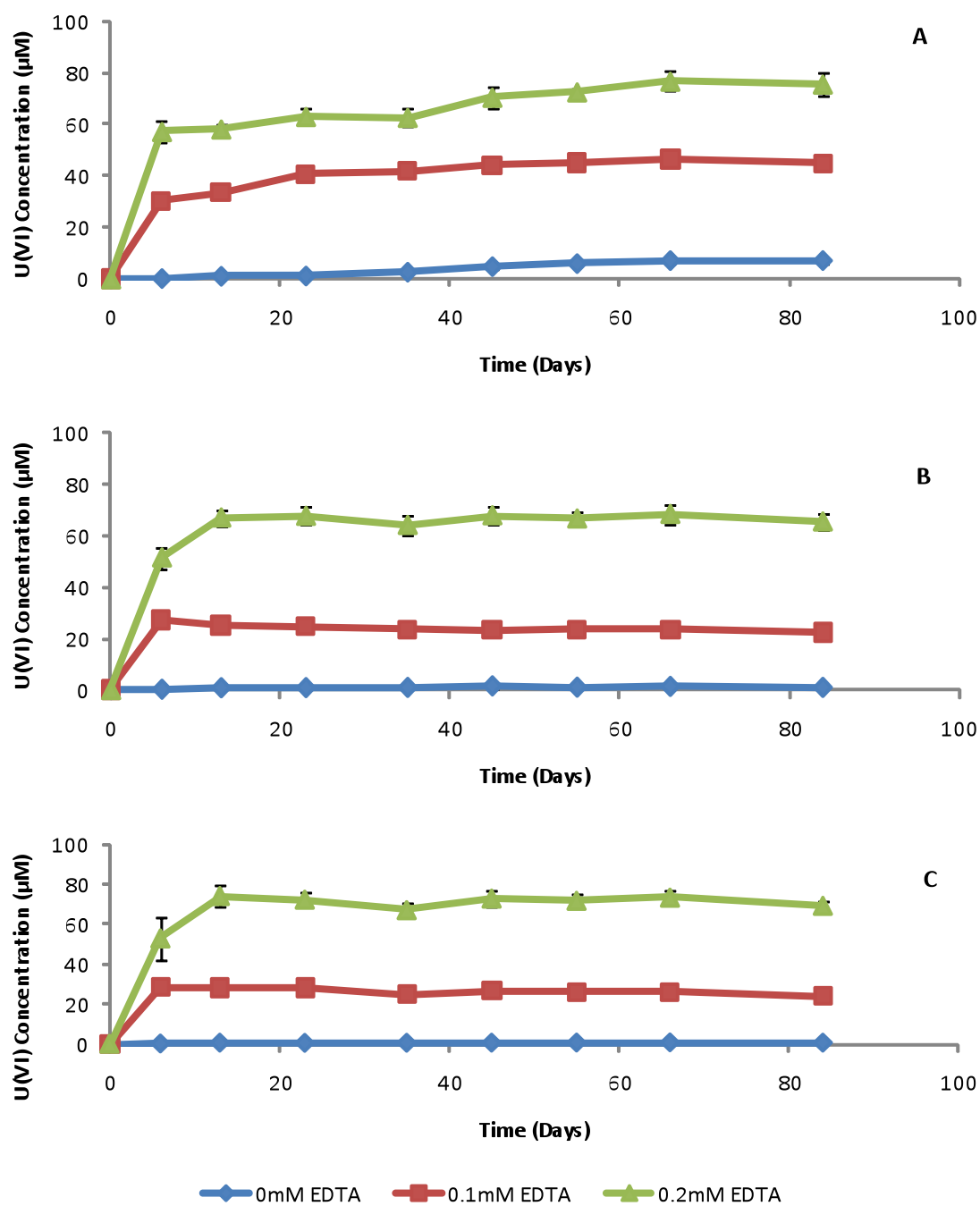


Figure 3.20. UO_2 reoxidation in the presence of EDTA and ferrihydrite (A), goethite (B), or hematite (C) with 10mM bicarbonate added showing EDTA promotes UO_2 reoxidation. Error bars represent the standard deviation of the triplicates.

U Reoxidation in the Presence of NTA and Fe(III) (hydr)oxides

Anaerobic batch systems containing bicarbonate, and ferrihydrite, goethite, or hematite were tested for UO_2 reoxidation in the presence of NTA (Figure 3.21). UO_2 reoxidation by NTA was less extensive than with EDTA, but more significant than with citrate. One possible explanation is NTA has a lower stability constant for Fe(III) compared to EDTA and higher compared to citrate. Also, NTA is unable to solubilize UO_2 (Figure 3.10), which was demonstrated with EDTA, potentially leading to less reoxidation. U(VI) values were highest with ferrihydrite compared to goethite and hematite, indicating that NTA promoted iron dissolution was an important mechanism as well as NTA enhanced extraction of adsorbed U(VI) on biotic UO_2 . After one week, U(VI) values were approximately $30\mu\text{M}$ with ferrihydrite, while both goethite and hematite were approximately $5\mu\text{M}$. In all the systems, more NTA present resulted in greater U(VI) values. Compared to EDTA, with a non-zero-order k_I between 0.044 and 0.049 day^{-1} for all Fe(III) (hydr)oxides, NTA had smaller reoxidation rates ($k_I = 0.026$, 0.0038 , and 0.004 day^{-1} for ferrihydrite, goethite, and hematite, respectively) but higher compared to citrate ($k_I = 0.009\text{ day}^{-1}$ for ferrihydrite) (Table 3.3).

The systems with NTA show more reoxidation with ferrihydrite than goethite or hematite resulting in the likely mechanism being NTA solubilizing iron, which reacts with UO_2 (Figure 3.21). NTA solubilized ferrihydrite resulting in U reoxidation, but was unable to solubilize goethite or hematite as effectively, leading to less reoxidation.

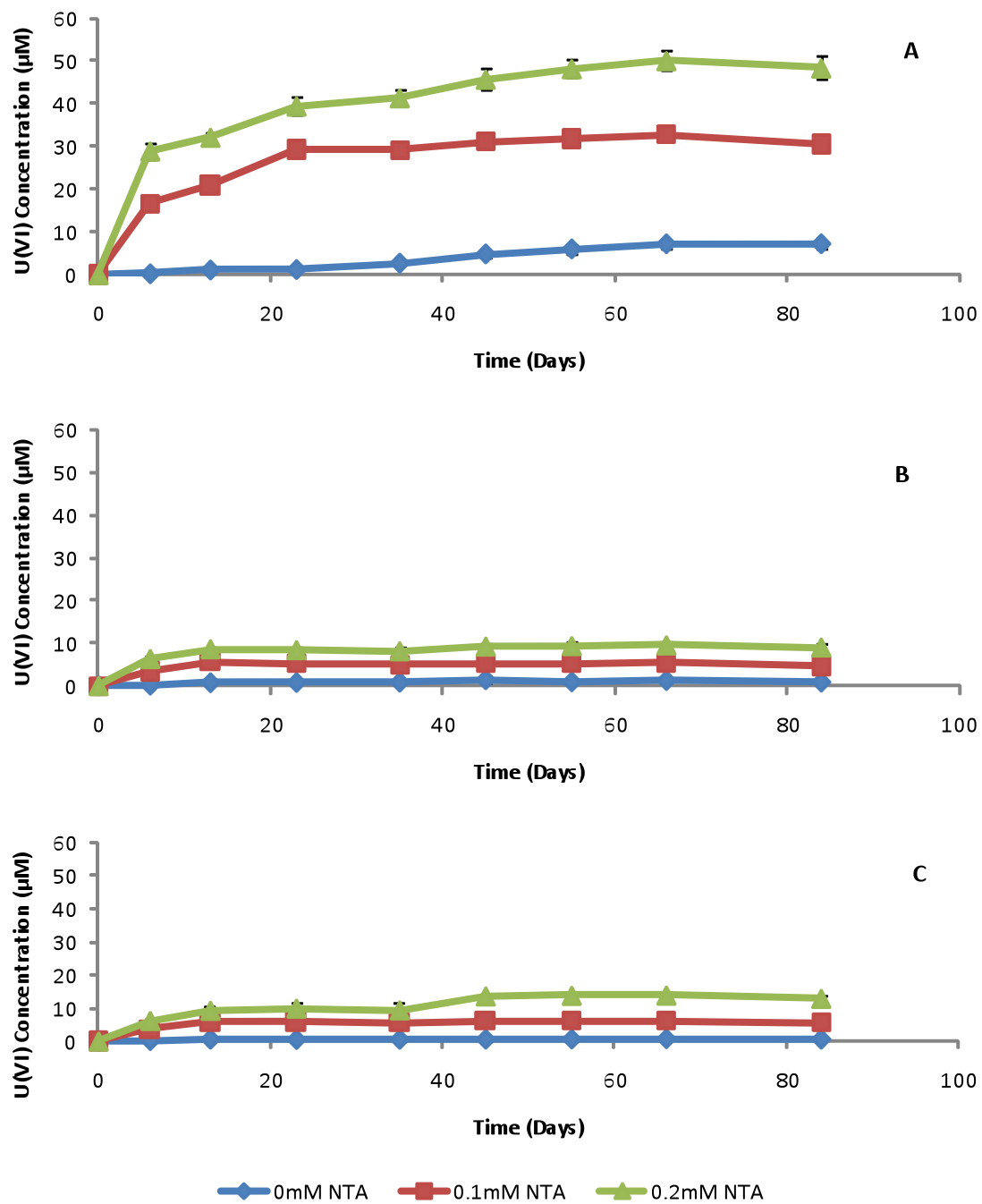


Figure 3.21. UO_2 reoxidation in the presence of NTA and ferrihydrite (A), goethite (B), or hematite (C) with 10mM bicarbonate added showing NTA promoted reoxidation. Error bars represent the standard deviation of the triplicates.

CHAPTER 4

CONCLUSIONS AND FUTURE WORK

Conclusions

Chelators have the ability to bind to both iron minerals and UO_2 and to increase dissolution rates, which may impact U subsurface fate by encouraging remobilization. The experiments performed demonstrate how chelators play a role in U stability. Microbially secreted chelators such as siderophores, or DFB in this case, show that DFB could affect U stability in U contaminated sites since DFB increased dissolution rates of iron minerals as well as extracted sorbed U(VI) . In contaminated settings, DFB could potentially be degraded leaving increased amounts of free Fe(III) to be available to reoxidize U very quickly as seen with Fe(III) added to UO_2 as ferric chloride. However, when DFB is bound to Fe(III) , UO_2 reoxidation is inhibited.

Another chelator potentially affecting U stability are organic molecules produced by microbial respiration, including citrate, which have the ability to chelate metals. Citrate increased UO_2 reoxidation rates with ferrihydrite ($k_1 = 0.009 \text{ day}^{-1}$ versus $k_1 = 0$ with goethite and hematite), but not the overall extent and Fe(III) bound to citrate could partially reoxidize UO_2 present. However, little UO_2 reoxidation was seen in systems with citrate and goethite or hematite. Citrate solubilized iron minerals, but was unable to solubilize biogenic UO_2 or extract adsorbed U(VI) from particles.

The chelator that caused the greatest amount of UO_2 to be reoxidized was EDTA followed by NTA. Both are synthetic chelators and are prevalent in the environment due

to industrial usage. EDTA enhanced Fe(III) reoxidation of UO_2 at the greatest rate out of all the chelators tested, which could be due to EDTA's ability to bind to both Fe(III) and U(IV) with moderately high stability constants. EDTA also solubilized all Fe(III) (hydr)oxides tested as well as biogenic UO_2 allowing for over half the total U available (approximately $80\mu\text{M}$) to be reoxidized within one week. Compared to EDTA, NTA was less effective in reoxidizing UO_2 , which can be explained by NTA's inability to solubilize UO_2 , as well as less dissolution of Fe(III) (hydr)oxides compared to EDTA. NTA was only able to reoxidize UO_2 in the presence of ferrihydrite through the dissolution of ferrihydrite and the extent of reoxidation was less compared to EDTA.

Chelators can affect U stability and there seems to be a general trend based on the stability constants with Fe(III), U(VI), and U(IV). The stability constant with DFB and Fe(III) was the highest ($\log K = 30.6$) and in this case, UO_2 reoxidation was inhibited with ferrihydrite and undetectable with goethite or hematite. DFB was also able to extract adsorbed U(VI) leading to a higher overall aqueous U(VI) concentration that could potentially drive U reoxidation. The lowest stability constant was with Fe(III) and citrate ($\log K = 11.19$) which the rate of UO_2 reoxidation was increased with ferrihydrite (non-zero-order $k_I = 0.009 \text{ day}^{-1}$) but not the extent ($< 15\mu\text{M}$). No UO_2 reoxidation occurred with goethite and hematite. The stability constants of EDTA or NTA with Fe(III) ($\log K$ of 25.1 and 16, respectively) are below DFB and above citrate. EDTA caused the greatest initial rate and extent of UO_2 reoxidation, non-zero-order $k_I = 0.049 \text{ day}^{-1}$ with ferrihydrite with $< 80\mu\text{M}$ U(VI) detected, and independent of the Fe(III) (hydr)oxide. NTA had the next highest rate of reoxidation with a non-zero-order $k_I =$

0.026 day⁻¹ with ferrihydrite with approximately 50 μM U(VI) detected. UO₂ reoxidation rates by NTA were much lower with goethite and hematite, non-zero-order $k_1 = 0.0038$ and 0.004 day⁻¹, respectively. It appears that the rate of dissolution of Fe(III) (hydr)oxides is an important mechanism when the chelator is unable to solubilize UO₂. However, in the case with EDTA, where the greatest UO₂ reoxidation occurred, EDTA-promoted dissolution of UO₂ is equally an important mechanism.

Many factors affect U stability, and the results shown in this chapter show how different types of chelators affect U stability by promoting dissolution of Fe(III) (hydr)oxides and/or biogenic UO₂. These results suggest that chelators as well as iron minerals play a key role in U mobilization and can potentially increase mobility and thus toxicity in contaminated settings where these chelators and minerals have been found. It is important to identify any patterns in U stability to ensure effective bioremediation.

Future Work

Immediate future work involves testing other Fe(III) chelators (shown in Table 4.1) in their ability to promote UO₂ and Fe(III) (hydr)oxide dissolution as well as their ability to reoxidize UO₂. Possible chelators that could be tested are siderophores that are tetradentate or bidentate and compare these results to DFB, which is hexadentate. Other chelators to test would be the ones that bind to Fe(III) less strongly than DFB but greater than EDTA. This would determine the maximum stability constant for Fe(III) and chelator that would not allow for UO₂ reoxidation. Chelators that can bind strongly to U(IV) should also be tested and compared to EDTA results. All chelators that can be found in natural settings should potentially be tested for their ability to reoxidize UO₂.

Table 4.1. Table of possible chelators to test for UO_2 reoxidation and their corresponding stability constant for Fe(III) (Boukhalfa and Crumbliss, 2002; NIST, 2004).

Chelator	log K for Fe(III)
Enterobactin	49
Desferrioxamine E	32.5
Pyoverdin	30.8
N-methylaceto	29.4
Aceto	28.29
DTPA	27.7
Aerobactin	22.5
EDDS	22
Oxalic Acid	18.49
L-Lysin hydroxamic acid	16.1

Also, other Fe(III) (hydr)oxides that are found in nature could also be tested in their role in UO_2 reoxidation with or without a chelator present. Other compounds besides chelators, such as those acting as electron shuttles (i.e. humic substances), could be tested for abiotic UO_2 reoxidation.

Differences in biogenic UO_2 and chemically precipitated UO_2 have been reported in the literature (Bargar et al., 2004; Fletcher et al., 2010). The experiments performed in this project could be conducted with chemically precipitated UO_2 as well as molecular U(IV) to observe any differences in UO_2 reoxidation rates. Washing steps for biogenic UO_2 with bicarbonate to remove adsorbed U(VI) could be affecting its reactivity, so a matrix of experiments could be set up containing freshly made biogenic UO_2 without washing in bicarbonate.

A set of biotic experiments could be conducted containing Fe(III) chelators with or without Fe(III) (hydr)oxides to test the effects microbes could play on UO_2 reoxidation rates. A multitude of these experiments could be conducted with various types of bacteria (examples include SRB and aerobic bacteria) that are able to reduce U(VI) to see

if reoxidation would occur. With the completion of these experiments, U stability will be better understood and will aid in clean up of U contaminated sites.

REFERENCES

- Abdelouas, A., Lutze, W., Gong, W., Nuttall, E. H., Strietelmeier, B. A., & Travis, B. J. (2000). Biological reduction of uranium in groundwater and subsurface soil. *The Science of the Total Environment* , 250, 21-35.
- Abdelouas, A., Yongming, L., Lutze, W., & Nuttall, H. E. (1998). Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated ground water. 35, 217-233.
- Archibald, F. (1983). *Lactobacillus plantarum*, an organism not requiring iron. *FEMS Microbiological Letters* , 19, 29-32.
- Atkinson, R. J., Posner, A. M., & Quirk, J. P. (1967). Adsorption of potential-determining ions at ferric oxide-aqueous electrolyte interface. *The Journal of Physical Chemistry* , 71, 550-558.
- Bargar, J. R., Bernier-Latmani, R., Giammar, D. E., & Tebo, B. M. (2008). Biogenic uraninite nanoparticles and their importance for uranium remediation. *Elements* , 4, 407-412.
- Beyenal, H., Sani, R. K., Peyton, B. M., Dohnalkova, A. C., Amonette, J. E., & Lewandowski, Z. (2004). Uranium immobilization by sulfate-reducing biofilms. *Environmental Science & Technology* , 2067-2074.
- Boukhalfa, H., & Crumbliss, A. L. (2002). Chemical aspects of siderophore mediated iron transport. *BioMetals* , 15, 325-339.
- Brainard, J. R., Strietelmeier, B. A., Smith, P. H., Langston-Unkefer, P. J., Barr, M. E., & Ryan, R. R. (1992). Actinide Binding and Solubilization by Microbial Siderophores. *Radiochimica acta* , 58-9, 357-363.
- Brooks, S. C., Fredrickson, J. K., Carroll, S. L., Kennedy, D. W., Zachara, J. M., Plymale, A. E., et al. (2003). Inhibition of bacterial U(VI) reduction by calcium. *Environmental Science and Technology* , 37, 1850-1858.
- Brooks, S. C., Taylor, D. L., & Jardine, P. M. (1996). Reactive transport of EDTA-complexed cobalt in the presence of ferrihydrite. *Geochimica et Cosmochimica Acta* , 60, 1899-1908.
- Bucheli-Witschel, M., & Egli, T. (2001). Environmental fate and microbial degradation of aminopolycarboxylic acids. *FEMS Microbiology Reviews* , 25, 69-106.
- Cheah, S.-F., Kraemer, S. M., Cervini-Silva, J., & Sposito, G. (2003). Steady-state Dissolution Kinetics of Goethite in the Presence of Desferrioxamine B and Oxalate Ligands: Implications for the Microbial Acquisition of Iron. *Chemical Geology* , 198, 63-75.

- Choppin, G. R., Erten, H. R., & Kia, Y. X. (1996). Variation of stability constants of thorium citrate complexes with ionic strength. *Radiochimica Acta* , 123-127.
- Cornell, R. M., & Schwertmann, U. (2003). *The Iron Oxides* (2nd Edition ed.). Weinheim, Germany: WILEY-VCH.
- Craft, E. S., Abu-Qare, A. W., Flaherty, M. M., Garofolo, M. C., Rincavage, H. L., & Abou-Donia, M. B. (2004). Depleted and natural uranium: Chemistry and toxicological effects. *Journal of Toxicology and Environmental Health* , 297-317.
- Duquene, L., Tack, F., Meers, E., Baeten, J., Wannijn, J., & Vandenhove, H. (2008). Effect of biodegradable amendments on uranium solubility in contaminated soils. *Science of the Total Environment* , 391, 26-33.
- Finneran, K. T., Housewright, M. E., & Lovley, D. R. (2002). Multiple influences of nitrate on uranium solubility during bioremediation of uranium-contaminated subsurface sediments. *Environmental Microbiology* , 4, 510-516.
- Fletcher, K. E., Boyanov, M. I., Thomas, S. H., Kemner, K. M., & Löffler, F. E. (2010). U(VI) reduction to mononuclear U(IV) by *Desulfitobacterium* species. *Environmental Science & Technology* , 44, 4705-4709.
- Frazier, S. W., Kretzschmar, R., & Kraemer, S. M. (2005). Bacterial Siderophores Promote Dissolution of UO₂ under Reducing Conditions. *Environmental Science & Technology* , 39, 5709-5715.
- Fredrickson, J. K., Zachara, J. M., Kennedy, D. W., Liu, C. G., Duff, M. C., Hunter, D. B., et al. (2002). Influence of Mn oxides on the reduction of uranium (VI) by the metal-reducing bacterium *Shewanella putrefaciens*. *Geochimica et Cosmochimica Acta* , 66 (18), 3247-3262.
- Gadd, G. M. (2000). Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Environmental Biotechnology* , 11, 271-279.
- Ganesh, R., Robinson, K. G., Chu, L., Kucsmas, D., & Reed, G. D. (1999). Reductive precipitation of uranium by *desulfovibrio desulfuricans*: Evaluation of cocontaminant effects and selective removal. *Water Research* , 33, 3447-3458.
- Ganesh, R., Robinson, K. G., Reed, G. D., & Sayler, G. S. (1997). Reduction of Hexavalent Uranium from Organic Complexes by Sulfate- and Iron-Reducing Bacteria. *Applied and Environmental Microbiology* , 4385-4391.
- Gavrilescu, M., Pavel, L. V., & Cretescu, I. (2009). Characterization and remediation of soils contaminated with uranium. *Journal of Hazardous Materials* (163), 175-510.

- Ginder-Vogel, M., Criddle, C. S., & Fendorf, S. (2006). Thermodynamic constraints on the oxidation of biogenic UO₂ by Fe(III) (Hydr)oxides. *Environmental Science and Technology* , 40, 3544-3550.
- Ginder-Vogel, M., Stewart, B., & Fendorf, S. (2010). Kinetic and mechanistic constraints on the oxidation of biogenic uraninite by ferrihydrite. *Environmental Science & Technology* , 44, 163-169.
- Gorby, Y. A., & Lovley, D. R. (1992). Enzymatic Uranium Precipitation. *Environmental Science and Technology* , 26, 205-207.
- Gu, B. H., Yan, H., Zhou, P., Watson, D. B., Park, M., & Istok, J. (2005). Natural humics impact uranium bioreduction and oxidation. *Environmental Science & Technology* , 39, 5268-5275.
- Guerinot, M. L. (1994). Microbial Iron Transport. *Annual Review of Microbiology* , 743-772.
- Hernlem, B. J., Vane, L. M., & Sayles, G. D. (1999). The Application of Siderophores for Metal Recovery and Waste Remediation: Examination of Correlations for Prediction of Metal Affinities. *Water Research* , 33 (4), 951-960.
- Hersman, L., Lloyd, T., & Sposito, G. (1995). Siderophore-promoted Dissolution of Hematite. *Geochimica et Cosmochimica Acta* , 59, 3327-3330.
- Hua, B., Xu, H., Terry, J., & Deng, B. (2006). Kinetics of uranium (VI) reduction by hydrogen sulfide in anoxic aqueous systems. *Environmental Science & Technology* , 40, 4666-4671.
- Jones, D. L. (1998). Organic acids in the rhizosphere- a critical review. *Plant and Soil* , 25-44.
- Kantar, C., Gillow, J. B., Harper-Arabie, R., Honeyman, B. D., & Francis, A. J. (2005). Determination of Stability Constants of U(VI)-Fe(III)-Citrate Complexes. *Environmental Science & Technology* , 2161-2168.
- Kraemer, S. M. (2004). Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Sciences* , 66, 3-18.
- Kraemer, S. M., Cheah, S.-F., Zapf, R., Xu, J., Raymond, K. N., & Sposito, G. (1999). Effect of Hydroxamate Siderophores on Fe Release and Pb(II) Adsorption by Goethite. *Geochimica et Cosmochimica Acta* , 63, 3003-3008.

- Langmuir, D. (1978). Uranium solution-mineral equilibria at low temperatures with applications to sedimentary ore deposits. *Geochimica et Cosmochimica Acta* , 42, 547-569.
- Liger, E., Charlet, L., & Cappellen, P. V. (1999). Surface catalysis of uranium (VI) reduction by iron (II). *Geochimica et Cosmochimica Acta* , 63, 2939-2955.
- Lloyd, J. R., & Lovley, D. R. (2001). Microbial detoxification of metals and radionuclides. *Environmental Biotechnology* , 12, 248-253.
- Lovley, D. R., & Coates, J. D. (1997). Bioremediation of metal contamination. *Environmental Biotechnology* , 8, 285-289.
- Lovley, D. R., & Phillips, E. J. (1992). Bioremediation of uranium contamination with enzymatic uranium reduction. *Environmental Science and Technology* , 26, 2228-2234.
- Lovley, D. R., Phillips, E. J., Gorby, Y. A., & Landa, E. R. (1991). Microbial Reduction of Uranium. *Nature* , 350, 413-416.
- Lovley, D. R., Roden, E. E., Phillips, E. J., & Woodward, J. C. (1993a). Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Marine Geology* , 113, 41-53.
- Lovley, D. R., Widman, P. K., Woodward, J. C., & Phillips, E. J. (1993b). Reaction of uranium by cytochrome c3 of desulfovibrio vulgaris. *Applied and Environmental Microbiology* , 59, 3572-3576.
- Means, J. L., Kucak, T., & Crear, A. (1980). Relative degradation rates of NTA, EDTA and DTPA and environmental applications. *Environmental Pollution* , 1, 45-60.
- Mullen, L., Gong, C., & Czerwinski, K. (2007). Complexation of uranium(VI) with the siderophore desferrioxamine B. *Journal of Radioanalytical and Nuclear Chemistry* , 273, 683-688.
- Neal, A. L., Techkarnjanaruk, S., Dohnalkova, A., McCready, D., Peyton, B. M., & Geesey, G. G. (2001). Iron sulfides and sulfur species produced at hematite surfaces in the presence of sulfate-reducing bacteria. *Geochimica Cosmochimica Acta* , 65, 223-235.
- Neilands, J. B. (1991). A Brief History of Iron Metabolism. *Biology of Metals* , 1-6.
- Neilands, J. B. (1993). Siderophores. *Archives of Biochemistry and Biophysics* , 302, 1-3.
- Nevin, K. P., & Lovley, D. R. (2000). Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments. *Environmental Science & Technology* , 34, 2472-2478.

- NIST, N. I. (2004, May). NIST Critically selected stability constants for metal complexes database, Version 8.0 for Windows, NIST standard reference database 46. U.S. Department of Commerce, Technology Administration, National institute of standards and technology . Gaithersburg, MD.
- Pasilis, S. P., & Pemberton, J. E. (2003). Speciation and coordination chemistry of uranyl(VI)-citrate complexes in aqueous solution. *Inorganic Chemistry* , 42, 6793-6800.
- Powell, P. E., Cline, G. R., Reid, P. P., & Szaniszlo, P. J. (1980). Occurrence of Hydroxamate Siderophore Iron Chelators in Soils. *Nature* , 287, 833-834.
- Reichard, P. U., Kretzschmar, R., & Kraemer, S. M. (2007). Dissolution Mechanisms of Goethite in the Presence of Siderophores and Organic Acids. *Geochimica et Cosmochimica Acta* , 71, 5635-5650.
- Richards, A. M. (2007). Identification and structural characterization of siderophores produced by halophilic and alkaliphilic bacteria. Washington State University.
- Sani, R. K., Peyton, B. M., Amonette, J. E., & Geesey, G. G. (2004). Reduction of uranium(VI) under sulfate-reducing conditions in the presence of Fe(III)-(hydr)oxides. *Geochimica et Cosmochimica Acta* , 68, 2639-2648.
- Sani, R. K., Peyton, B. M., Dohnalkova, A., & Amonette, J. E. (2005). Reoxidation of reduced uranium with iron(III) (hydr)oxides under sulfate-reducing conditions. *Environmental Science & Technology* (39), 2059-2066.
- Schwertmann, U., & Cornell, R. M. (2000). *Iron oxides in the laboratory* (2nd Edition ed.). New York: Wiley-VCH.
- Siebner-Freibach, H., Hadar, Y., Chen, Y. (2004). Interaction of iron chelating agents with clay minerals. *Soil Science Society of America* , 68, 470-480.
- Senko, J. M., Mohamed, Y. D., & Krumholz, L. R. (2005a). Role for Fe(III) minerals in nitrate-dependent microbial U(IV) oxidation. *Environmental Science & Technology* , 39, 2529-2536.
- Senko, J. M., Mohamed, Y., Dewers, T., & Krumholz, L. R. (2005b). Role for Fe(III) minerals in nitrate-dependent microbial U(IV) oxidation. *Environmental Science & Technology* , 39, 2529-2536.
- Senko, J. M., Suflita, J. M., & Krumholz, L. R. (2005c). Geochemical controls on microbial nitrate-dependent U(IV) oxidation. *Geomicrobiology Journal* , 22, 371-378.

- Suzuki, Y., Kelly, S. D., Kemner, K. M., & Banfield, J. F. (2003). Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. *Applied and Environmental Microbiology*, 69 (3), 1337-1346.
- Suzuki, Y., Tanaka, K., Kozai, N., & Ohnuki, T. (2010). Effects of Citrate, NTA, and EDTA on the Reduction of U(VI) by *Shewanella putrefaciens*. *Geomicrobiology Journal*, 245-250.
- Tebo, B. M., & Obraztsova, A. Y. (1998). Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiology Letters*, 162, 193-198.
- Touati, D. (2000). Iron and oxidative stress in bacteria. *Archives of Biochemistry and Biophysics*, 373 (1), 1-6.
- Ulrich, K. U., Ilton, E. S., Veeramani, H., Sharp, J. O., Bernier-Latmani, R., Schofield, E. J., Bargar, J. R., Glammar, D. E. (2009). Comparative dissolution kinetics of biogenic and chemogenic uraninite under oxidizing conditions in the presence of carbonate. *Geochimica et Cosmochimica Acta*, 73 (20), 6065-6083.
- Wan, J., Tokunaga, T., Brodie, E., Wang, Z., Zheng, Z., Herman, D., Hazen, T. C., Firestone, M. K., Sutton, S. R. (2005). Reoxidation of bio-reduced uranium under reducing conditions. *Environmental Science and Technology*, 39, 6162-6169.
- Wandersman, C., & Delepelaire, P. (2004). Bacterial Iron Sources: From siderophores to hemophores. *Annual Review of Microbiology*, 58, 611-647.
- Wersin, P., Hochella, M. F., Persson, P., Redden, G., Lecie, J. O., & Harris, D. W. (1994). Interactions between aqueous uranium (VI) and sulfide minerals: Spectroscopic evidence for sorption and reduction. *Geochimica et Cosmochimica Acta*, 58 (13), 2829-2843.
- Winkelman, G. (1991). *Handbook of Microbial Iron Chelates*. Boca Raton: CRC Press.

APPENDICES

APPENDIX A

RAW DATA FOR UO_2 DISSOLUTION EXPERIMENTS

Figure A1. A KPA-11 calibration curve for U(VI) using manual mode. All calibration curves were similar to this and blanks had typical intercept values < 3000 for the low curve.

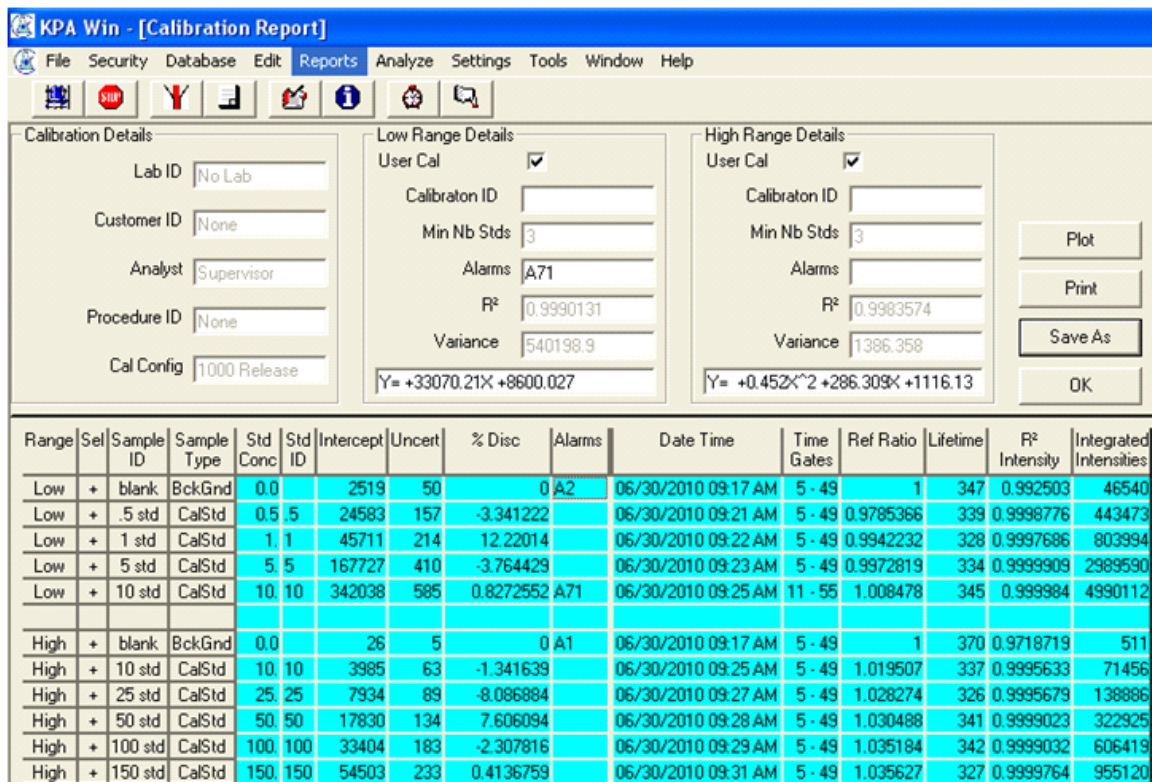


Figure A2. A KPA-11 calibration curve for total dissolved U using automatic mode. All calibration curves were similar to this and blanks had typical intercept values < 3000 for the low curve.

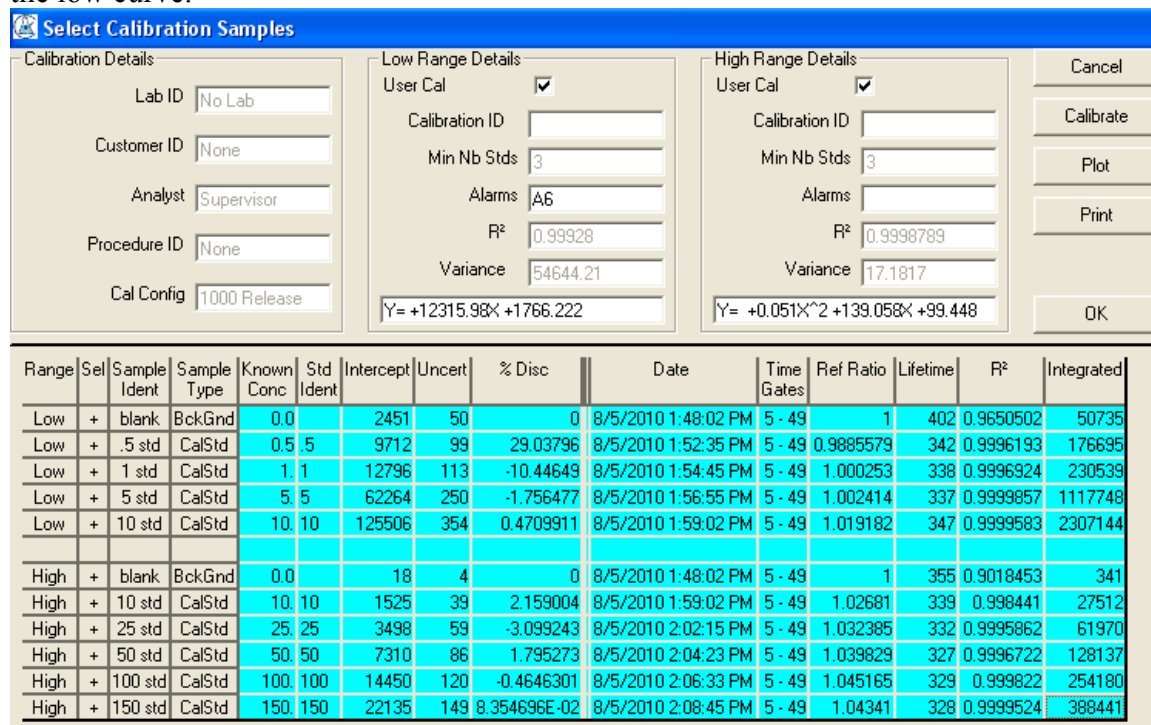


Table A1. Raw data for UO_2 dissolution in the presence of DFB at 0mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	DFB Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
12/15/09	0	0	1	0.929	0.659	2.636
			2	1.055	0.693	2.772
			3	1.134	0.624	2.496
		0.1	1	1.023	0.667	2.668
			2	0.948	0.637	2.548
			3	0.918	0.631	2.524
		0.2	1	2.347	0.634	2.536
			2	1.811	2.46	9.84
			3	2.909	0.649	2.596
12/16/09	1	0	1	1.107	0.926	3.704
			2	1.302	0.934	3.736
			3	1.036	0.864	3.456
		0.1	1	0.963	0.871	3.484
			2	0.897	0.815	3.26
			3	0.911	0.84	3.36
		0.2	1	0.914	0.863	3.452
			2	1.012	0.945	3.78
			3	1.054	0.923	3.692
12/18/09	3	0	1	0.909	0.432	1.728
			2	1.042	0.615	2.46
			3	0.75	0.56	2.24
		0.1	1	0.701	0.408	1.632
			2	0.601	0.183	0.732
			3	0.725	0.26	1.04
		0.2	1	0.663	0.246	0.984
			2	0.678	0.28	1.12
			3	0.755	0.277	1.108
12/21/09	6	0	1	0.704	0.379	1.516
			2	1.107	0.449	1.796
			3	0.788	0.398	1.592
		0.1	1	3.047	0.397	1.588
			2	0.685	0.381	1.524
			3	0.67	0.434	1.736
		0.2	1	0.745	0.434	1.736
			2	0.803	0.456	1.824
			3	0.906	0.459	1.836
1/5/10	21	0	1	0.988	0.447	1.788
			2	1.253	0.583	2.332
			3	0.875	0.485	1.94
		0.1	1	1.557	0.863	3.452
			2	1.548	0.697	2.788
			3	1.551	0.677	2.708
		0.2	1	1.874	1.095	4.38
			2	1.693	0.787	3.148
			3	1.795	0.766	3.064

Table A2. Raw data for UO_2 dissolution in the presence of DFB at 3mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	DFB Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
1/21/10	0	0	1	1.077	0.206	0.824
			2	0.546	0.476	1.904
			3	0.411	0.279	1.116
		0.1	1	0.361	0.175	0.7
			2	2.266	0.169	0.676
			3	0.359	0.17	0.68
		0.2	1	1.199	0.194	0.776
			2	0.373	0.238	0.952
			3	0.342	0.548	2.192
1/24/10	3	0	1	0.456	0.093	0.372
			2	0.444	0.098	0.392
			3	4.046	0.109	0.436
		0.1	1	0.648	0.135	0.54
			2	0.602	0.145	0.58
			3	1.326	0.149	0.596
		0.2	1	0.719	0.171	0.684
			2	0.743	0.188	0.752
			3	0.711	0.425	1.7
1/27/10	6	0	1	1.55	0.184	0.736
			2	0.884	0.145	0.58
			3	0.745	0.169	0.676
		0.1	1	0.644	0.64	2.56
			2	0.7	0.224	0.896
			3	0.797	0.201	0.804
		0.2	1	3.778	0.244	0.976
			2	0.992	0.245	0.98
			3	1.048	0.38	1.52
1/30/10	9	0	1	0.302	0.235	0.94
			2	0.23	0.92	3.68
			3	0.461	0.188	0.752
		0.1	1	0.522	0.309	1.236
			2	1.247	0.373	1.492
			3	1.265	0.341	1.364
		0.2	1	0.582	0.408	1.632
			2	0.637	0.475	1.9
			3	0.647	0.48	1.92
2/2/10	12	0	1	0.475	0.093	0.372
			2	0.382	0.123	0.492
			3	2.197	0.64	2.56
		0.1	1	1.415	0.217	0.868
			2	1.867	0.27	1.08
			3	1.51	0.255	1.02
		0.2	1	0.797	0.367	1.468
			2	0.893	0.369	1.476
			3	0.892	0.333	1.332
2/5/10	15	0	1	0.418	0.16	0.64
			2	0.499	0.148	0.592
			3	0.309	0.153	0.612
		0.1	1	0.797	0.292	1.168
			2	0.64	0.298	1.192
			3	0.679	0.314	1.256
		0.2	1	0.829	0.406	1.624
			2	1.615	0.444	1.776
			3	0.951	0.44	1.76
2/8/10	18	0	1	0.687	0.152	0.608
			2	2.903	0.154	0.616
			3	0.751	0.153	0.612

Table A2. Continued

Date	Days	DFB Conc. (mM)		U(VI) (μ M)	KPA U(tot) aq. (μ M)	U(tot) aq. (μ M)
2/11/10	21	0.1	1	1.104	0.315	1.26
			2	1.22	0.317	1.268
			3	1.083	0.306	1.224
		0.2	1	1.162	0.401	1.604
			2	1.139	0.476	1.904
			3	5.55	0.48	1.92
		0	1	2.939	0.137	0.548
			2	0.014	0.119	0.476
			3	0.089	0.114	0.456
		0.1	1	0.409	0.302	1.208
			2	0.442	0.323	1.292
			3	0.453	0.312	1.248
2/25/10	35	0.2	1	0.668	0.43	1.72
			2	0.908	0.514	2.056
			3	0.902	0.463	1.852
		0	1	1.805	0.25	1
			2	0.352	0.177	0.708
			3	0.371	0.169	0.676
		0.1	1	1.246	0.452	1.808
			2	1.207	0.793	3.172
			3	1.281	0.481	1.924
		0.2	1	2.062	0.727	2.908
			2	2.731	1.086	4.344
			3	3.281	0.808	3.232
5/26/10	125	0	1	0.757	0.036	0.144
			2	0.261	0	0
			3	0.17	0	0
		0.1	1	4.074	1.003	4.012
			2	4.079	1.141	4.564
			3	8.605	1.054	4.216
		0.2	1	9.74	2.221	8.884
			2	9.508	2.895	11.58
			3	9.949	2.863	11.452

Table A3. Raw data for UO_2 dissolution in the presence of DFB at 10mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	DFB Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
1/21/10	0	0	1	0.277	0.125	0.5
			2	0.277	0.163	0.652
			3	0.34	0.162	0.648
		0.1	1	0.4	0.139	0.556
			2	0.409	0.172	0.688
			3	0.341	0.229	0.916
		0.2	1	0.374	0.169	0.676
			2	0.403	0.303	1.212
			3	6.341	0.145	0.58
1/24/10	3	0	1	0.965	0.196	0.784
			2	1.238	0.285	1.14
			3	1.3	0.277	1.108
		0.1	1	1.01	0.184	0.736
			2	0.731	0.209	0.836
			3	0.769	0.223	0.892
		0.2	1	0.781	0.272	1.088
			2	0.783	0.281	1.124
			3	0.744	0.238	0.952
1/27/10	6	0	1	1.079	0.246	0.984
			2	1.091	0.274	1.096
			3	1.167	0.33	1.32
		0.1	1	0.902	0.275	1.1
			2	0.956	0.268	1.072
			3	1.279	0.298	1.192
		0.2	1	0.983	0.323	1.292
			2	0.953	0.451	1.804
			3	0.961	0.337	1.348
1/30/10	9	0	1	0.992	0.409	1.636
			2	0.863	0.405	1.62
			3	1.144	0.357	1.428
		0.1	1	0.658	0.342	1.368
			2	0.772	0.378	1.512
			3	0.739	0.386	1.544
		0.2	1	1.378	0.462	1.848
			2	0.937	0.685	2.74
			3	1.463	0.473	1.892
2/2/10	12	0	1	1.124	0.138	0.552
			2	1.024	0.161	0.644
			3	1.201	0.315	1.26
		0.1	1	1.014	0.265	1.06
			2	0.869	0.35	1.4
			3	0.956	0.35	1.4
		0.2	1	1.133	0.392	1.568
			2	1.094	0.635	2.54
			3	1.066	0.372	1.488
2/5/10	15	0	1	1.062	0.253	1.012
			2	0.972	0.294	1.176
			3	1.024	0.357	1.428
		0.1	1	1.068	0.278	1.112
			2	0.967	0.408	1.632
			3	1.293	0.431	1.724
		0.2	1	1.274	0.526	2.104
			2	1.23	0.504	2.016
			3	1.248	0.524	2.096
2/8/10	18	0	1	1.444	0.219	0.876
			2	1.041	0.274	1.096
			3	1.002	0.264	1.056

Table A3. Continued

Date	Days	DFB Conc. (mM)		U(VI) (μ M)	KPA U(tot) aq. (μ M)	U(tot) aq. (μ M)
2/11/10	21	0.1	1	1.124	0.248	0.992
			2	1.775	0.422	1.688
			3	1.19	0.426	1.704
		0.2	1	1.513	0.514	2.056
			2	1.526	0.554	2.216
			3	1.602	0.56	2.24
		0	1	0.825	0.207	0.828
			2	0.792	0.322	1.288
			3	0.796	0.344	1.376
		0.1	1	0.651	0.411	1.644
			2	0.532	0.435	1.74
			3	0.004	0.459	1.836
2/25/10	35	0.2	1	0.926	0.568	2.272
			2	1.224	0.599	2.396
			3	1.16	0.603	2.412
		0	1	1.457	0.368	1.472
			2	1.221	0.388	1.552
			3	1.843	0.417	1.668
		0.1	1	2.024	0.567	2.268
			2	2.071	1.435	5.74
			3	2.159	0.719	2.876
		0.2	1	3.259	1.021	4.084
			2	3.158	1.027	4.108
			3	3.32	1.178	4.712
5/26/10	125	0	1	1.285	0.173	0.692
			2	1.144	0.121	0.484
			3	1.042	0.165	0.66
		0.1	1	6.988	1.696	6.784
			2	7.069	1.904	7.616
			3	7.111	2.218	8.872
		0.2	1	14.565	3.706	14.824
			2	15.158	4.386	17.544
			3	14.894	4.263	17.052

Table A4. Raw data for UO_2 dissolution in the presence of citrate at 0mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	Citrate Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
4/21/10	0	0	1		0	0
			2		0	0
			3		0	0
		0.1	1		0.004	0.016
			2		0.014	0.056
			3		0.01	0.04
		0.2	1		0.016	0.064
			2		0.055	0.22
			3		0	0
4/26/10	7	0	1	0.027	0	0
			2	0.058	0	0
			3	0	0	0
		0.1	1	0.197	0	0
			2	0.195	0	0
			3	0.07	0	0
		0.2	1	0.636	0.032	0.128
			2	1.116	0.224	0.896
			3	0.552	0.176	0.704
5/3/10	14	0	3	0.794	0.45	1.8
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
5/10/10	21	0	3	0	0	0
			1	0.093	0	0
			2	0.119	0	0
		0.1	3	0.159	0	0
			1	0.142	0	0
			2	0.075	0	0
		0.2	3	0.173	0	0
			1	0.132	0	0
			2	0	0	0
5/22/10	33	0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
6/1/10	43	0	3	0	0	0
			1	0	0.01	0.04
			2	0	0	0
		0.1	3	0.036	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
6/12/10	53	0	3	0	0	0
			1	0	0	0
			2	0	0	0

Table A4. Continued

Date	Days	Citrate Conc. (mM)		U(VI) (μ M)	KPA U(tot) aq. (μ M)	U(tot) aq. (μ M)
6/22/10	63	0.1	3	0.557	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
		0	3	0	0	0
			1	0.009	0	0
			2	0.053	0	0
		0.1	3	0.152	0	0
			1	0.002	0	0
			2	0.309	0	0
7/2/10	73	0.2	3	0.126	0	0
			1	0.126	0	0
			2	0.001	0	0
		0	3	0.115	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
7/14/10	85	0	3	0	0	0
			1	0.686	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
			3	0	0	0

Table A5. Raw data for UO_2 dissolution in the presence of citrate at 3mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	Citrate Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
4/21/10	0	0	1		0	0
			2		0	0
			3		0	0
		0.1	1		0	0
			2		0.006	0.024
			3		0	0
		0.2	1		0	0
			2		0	0
			3		0	0
4/26/10	7	0	1	0.006	0	0
			2	0	0	0
			3	0	0	0
		0.1	1	0.49	0.03	0.12
			2	0.602	0.139	0.556
			3	0.098	0.01	0.04
		0.2	1	0.647	0.007	0.028
			2	0.609	0.152	0.608
			3	0.691	0.161	0.644
5/3/10	14	0	1	0	0	0
			2	0	0	0
			3	0	0	0
		0.1	1	0	0	0
			2	0	0	0
			3	0	0	0
		0.2	1	0	0	0
			2	0	0	0
			3	0	0	0
5/10/10	21	0	1	0	0	0
			2	0	0	0
			3	0	0	0
		0.1	1	0	0	0
			2	0	0	0
			3	0.056	0	0
		0.2	1	0.024	0	0
			2	0.007	0	0
			3	0.037	0	0
5/22/10	33	0	1	0	0	0
			2	0	0	0
			3	0	0	0
		0.1	1	0.014	0	0
			2	0.121	0	0
			3	0	0	0
		0.2	1	0	0	0
			2	0	0	0
			3	0	0	0
6/1/10	43	0	1	0	0	0
			2	0	0	0
			3	0	0	0

Table A5. Continued

Date	Days	Citrate Conc. (mM)		U(VI) (μ M)	KPA U(tot) aq. (μ M)	U(tot) aq. (μ M)
6/12/10	53	0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
		0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
6/22/10	63	0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
		0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
7/2/10	73	0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
		0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
7/14/10	85	0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.2	3	0	0	0
			1	0	0	0
			2	0	0	0
		0	3	0	0	0
			1	0	0	0
			2	0	0	0
		0.1	3	0	0	0
			1	0	0	0
			2	0	0	0

Table A6. Raw data for UO_2 dissolution in the presence of citrate at 10mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	Citrate Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
4/21/10	0	0	1		0.061	0.244
			2		0.076	0.304
			3		0.092	0.368
		0.1	1		0.15	0.6
			2		0.029	0.116
			3		0.1	0.4
		0.2	1		0.629	2.516
			2		0.686	2.744
			3		0.596	2.384
4/26/10	7	0	1	0.299	0	0
			2	0.206	0.024	0.096
			3	0.245	0.047	0.188
		0.1	1	1.189	0.175	0.7
			2	0.543	0.125	0.5
			3	0.733	0.216	0.864
		0.2	1	1.447	0.05	0.2
			2	1.027	0.305	1.22
			3	0.23	0	0
5/3/10	14	0	1	0.23	0	0
			2	0.145	0	0
			3	0.141	0	0
		0.1	1	2.466	0	0
			2	0.29	0	0
			3	0.301	0	0
		0.2	1	0.338	0.029	0.116
			2	0.497	0.01	0.04
			3	0.2	0.004	0.016
5/10/10	21	0	1	0.256	0	0
			2	0.387	0.002	0.008
			3	0.368	0.03	0.12
		0.1	1	0.27	0.059	0.236
			2	0.132	0.019	0.076
			3	0.15	0.012	0.048
		0.2	1	0.176	0.016	0.064
			2	0.059	0.018	0.072
			3	0.006	0	0
5/22/10	33	0	1	0.156	0.048	0.192
			2	0.161	0.059	0.236
			3	0.218	0.067	0.268
		0.1	1	0.276	0.054	0.216
			2	0.116	0.073	0.292
			3	0.226	0.052	0.208
		0.2	1	0.105	0.024	0.096
			2	0.105	0.045	0.18
			3	0.023	0.042	0.168
6/1/10	43	0	1	0.012	0.006	0.024
			2	0.096	0.038	0.152
			3	0.065	0.042	0.168

Table A6. Continued

Date	Days	Citrate Conc. (mM)		U(VI) (μ M)	KPA U(tot) aq. (μ M)	U(tot) aq. (μ M)
6/12/10	53	0.1	1	0.147	0.025	0.1
			2	0.12	0.036	0.144
			3	0.335	0.042	0.168
		0.2	1	0.245	0.017	0.068
			2	0.026	0.033	0.132
			3	0	0.011	0.044
		0	1	0.001	0	0
			2	0.071	0	0
			3	0.119	0	0
		0.1	1	0.133	0	0
			2	0.002	0	0
			3	0.065	0	0
6/22/10	63	0.2	1	0.014	0	0
			2	0	0	0
			3	0	0	0
		0	1	0.092	0	0
			2	0.221	0	0
			3	0.19	0	0
		0.1	1	0.264	0	0
			2	0.17	0	0
			3	0.174	0	0
		0.2	1	0.121	0	0
			2	0.504	0	0
			3	0.055	0	0
7/2/10	73	0	1	0.037	0	0
			2	0.106	0.067	0.268
			3	0.065	0.076	0.304
		0.1	1	0.133	0.057	0.228
			2	0	0.075	0.3
			3	0.111	0.095	0.38
		0.2	1	0	0	0
			2	0.076	0	0
			3	0	0	0
		0	1	0	0	0
			2	0.022	0	0
			3	0	0	0
7/14/10	85	0.1	1	0.026	0	0
			2	0	0	0
			3	0	0	0
		0.2	1	0	0	0
			2	0	0	0
			3	0	0	0
		0	1	0	0	0
			2	0	0	0
			3	0	0	0

Table A7. Raw data for UO_2 dissolution in the presence of EDTA at 10mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	EDTA Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
6/2/10	0	0	1	0	0	0
			2	0	0.002	0.008
			3	0	0	0
		0.1	1	0	0	0
			2	0	0	0
			3	0	0	0
		0.2	1	0.119	0.039	0.156
			2	0	0	0
			3	0	0	0
6/11/10	9	0	1	0.507	0	0
			2	0.457	0	0
			3	0.378	0	0
		0.1	1	0.631	0.231	0.924
			2	0.796	0.648	2.592
			3	0.354	0.617	2.468
		0.2	1	1.319	0.391	1.564
			2	0.768	0.713	2.852
			3	0.739	0.792	3.168
6/21/10	19	0	1	0.366	0	0
			2	0.323	0	0
			3	0.22	0	0
		0.1	1	1.707	0.084	0.336
			2	2.253	0.614	2.456
			3	1.244	0.584	2.336
		0.2	1	4.504	0.266	1.064
			2	2.785	0.951	3.804
			3	2.817	0.982	3.928
7/2/10	30	0	1	0.197	0.077	0.308
			2	0.357	0.137	0.548
			3	0.13	0.105	0.42
		0.1	1	0.86	0.596	2.384
			2	1.222	0.996	3.984
			3	0.748	0.828	3.312
		0.2	1	3.071	0.919	3.676
			2	1.809	1.366	5.464
			3	3.606	1.374	5.496
7/14/10	42	0	1	0.08	0	0
			2	0.122	0	0
			3	0.016	0	0
		0.1	1	0.773	0.412	1.648
			2	1.149	0.838	3.352
			3	0.75	0.8	3.2
		0.2	1	2.804	0.63	2.52
			2	1.639	1.403	5.612
			3	2.036	1.361	5.444
7/25/10	53	0	1	0.128	0	0
			2	0.196	0	0
			3	0	0	0
		0.1	1	1.007	0.282	1.128
			2	1.518	0.59	2.36
			3	0.747	0.608	2.432
		0.2	1	3.561	0.663	2.652
			2	2.078	1.072	4.288
			3	2.504	1.209	4.836

Table A8. Raw data for UO_2 dissolution in the presence of NTA at 10mM bicarbonate showing KPA-11 data for U(VI) and total aqueous U concentrations.

Date	Days	NTA Conc. (mM)		U(VI) (μM)	KPA U(tot) aq. (μM)	U(tot) aq. (μM)
6/2/10	0	0	1	0	0	0
			2	0	0.002	0.008
			3	0	0	0
		0.1	1	0.005	0.003	0.012
			2	0	0	0
			3	0.061	0	0
		0.2	1	0	0	0
			2	0.077	0.006	0.024
			3	0	0.002	0.008
6/11/10	9	0	1	0.507	0	0
			2	0.457	0	0
			3	0.378	0	0
		0.1	1	0.465	0.028	0.112
			2	1.036	0.312	1.248
			3	0.562	0.157	0.628
		0.2	1	0.216	0	0
			2	0.572	0.134	0.536
			3	0.887	0.244	0.976
6/21/10	19	0	1	0.366	0	0
			2	0.323	0	0
			3	0.22	0	0
		0.1	1	1.102	0	0
			2	1.767	0.256	1.024
			3	1.062	0.1	0.4
		0.2	1	0.596	0	0
			2	1.506	0.195	0.78
			3	1.592	0.196	0.784
7/2/10	30	0	1	0.197	0.077	0.308
			2	0.357	0.137	0.548
			3	0.13	0.105	0.42
		0.1	1	1.379	0.25	1
			2	2.091	0.658	2.632
			3	1.033	0.357	1.428
		0.2	1	0.888	0.169	0.676
			2	2.227	0.64	2.56
			3	2.137	0.662	2.648
7/14/10	42	0	1	0.08	0	0
			2	0.122	0	0
			3	0.016	0	0
		0.1	1	1.089	0.068	0.272
			2	1.599	0.503	2.012
			3	0.779	0.243	0.972
		0.2	1	0.554	0	0
			2	1.696	0.472	1.888
			3	1.431	0.527	2.108
7/25/10	53	0	1	0.128	0	0
			2	0.196	0	0
			3	0	0	0
		0.1	1	1.317	0.071	0.284
			2	1.804	0.346	1.384
			3	0.921	0.162	0.648
		0.2	1	0.711	0.035	0.14
			2	2.106	0.387	1.548
			3	1.673	0.341	1.364

APPENDIX B

RAW DATA FOR UO_2 REOXIDATION EXPERIMENTS

Table B1. Raw data for the reoxidation of UO_2 in the presence of DFB with goethite (G) or hematite (H) at 0mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Iron oxide	DFB Conc. (mM)		U(VI) (μM)	Date	Days	Iron oxide	DFB Conc. (mM)		U(VI) (μM)
2/5/10	0	G	0	1	0.157	2/18/10	13	G	0	1	0.066
				2	0.03					2	0.02
				3	0.047					3	0.099
			0.1	1	0.066				0.1	1	0.339
				2	0.004					2	0.565
				3	0.053					3	0.545
			0.2	1	0.409				0.2	1	0.719
				2	0.017					2	0.673
				3	0.304					3	0.76
		H	0	1	0.213			H	0	1	0.087
				2	0.264					2	0.152
				3	0.096					3	0.099
			0.1	1	0.395				0.1	1	0.456
				2	3.799					2	0.447
				3	0.692					3	0.449
			0.2	1	0.048				0.2	1	0.501
				2	0.03					2	0.69
				3	0.551					3	0.312
2/7/10	2	G	0	1	2.175	2/23/10	18	G	0	1	0.235
				2	0.067					2	0.26
				3	0.271					3	0.274
			0.1	1	0.385				0.1	1	0.299
				2	1.254					2	0.561
				3	0.397					3	0.68
			0.2	1	0.746				0.2	1	0.203
				2	0.474					2	0.192
				3	0.685					3	0.353
		H	0	1	0.125			H	0	1	0.217
				2	1.572					2	0.15
				3	0.132					3	0.484
			0.1	1	0.396				0.1	1	0.366
				2	0.562					2	4.452
				3	0.354					3	0.645
			0.2	1	0.525				0.2	1	0.912
				2	0.358					2	1.007
				3	0.469					3	1.291
2/9/10	4	G	0	1	0.107	3/1/10	25	G	0	1	0
				2	0.192					2	
				3	0.031					3	0
			0.1	1	0.237				0.1	1	0.154
				2	0.199					2	0.428
				3	0.323					3	0.494
			0.2	1	0.352				0.2	1	0.663
				2	0.294					2	1.229
				3	0.299					3	1.154
		H	0	1	0			H	0	1	0

Table B1. Continued

Table 2-1. Continued													
Date	Days	Iron oxide	DFB Conc. (mM)	U(VI) (μ M)	Date	Days	Iron oxide	DFB Conc. (mM)	U(VI) (μ M)				
2/11/10	6	G	0	2	0.085	3/10/10	34	G	0	2	0		
				3	0.05					3	0		
				0.1	1					0.378	0.1	1	0
					2					0.244		2	0
					3					0.222		3	0.144
					0.2					1		0	0.2
			2						0.324	2		1.171	
			3						0.546	3		1.318	
			0	1	0.058				0	1	0.18		
				2	0.04					2	0.056		
				3	0.052					3	0.409		
				0.1	1					0.259	0.1	1	0.932
					2					0.26		2	0.812
					3					0.392		3	0.827
			0.2	1	0.502				0.2	1	1.669		
				2	0.438					2	1.71		
				3	0.092					3	1.713		
				H	0					1	0.034	H	0
		2						0.496		2	0.084		
		3						0.032		3	0.025		
		0.1	1		0.316			0.1	1	0.188			
			2		0.169				2	0.476			
			3		0.195				3	0.685			
		0.2	1	0.415	0.2			1	1.531				
			2	0.397				2	1.862				
			3	0.593				3	3.495				
2/13/10	8		G	0		1	0.034	3/31/10	55	G	0	1	0.106
						2	0.021					2	0.014
						3	0					3	0.031
		0.1		1	0.383	0.1	1				0.472		
				2	0.335		2				1.252		
				3	0.423		3				1.084		
		0.2	1	0.462	0.2	1	2.624						
			2	0.358		2	2.667						
			3	0.423		3	2.798						
		H	0	1	0.04	H	0			1	0.199		
				2	0.017					2	0		
				3	0.18					3	0.025		
0.1	1		0.417	0.1	1		0.232						
	2		0.363		2		0.326						
	3		0.418		3		0.626						
0.2	1	0.559	0.2	1	2.486								
	2	0.481		2	2.867								
	3	0.592		3	2.973								
2/15/10	10	G	0	1	0.025	5/18/10	103	G	0	1	0.086		
				2	0.104					2	0.06		
				3	0.04					3	0.401		

Table B1. Continued

Date	Days	Iron oxide	DFB Conc. (mM)	U(VI) (μ M)	Date	Days	Iron oxide	DFB Conc. (mM)	U(VI) (μ M)
			0.1	1 0.343				0.1	1 1.322
				2 0.292					2 1.479
				3 0.27					3 1.375
			0.2	1 0.457				0.2	1 4.403
				2 0.473					2 5.077
				3 0.427					3 4.852
		H	0	1 0.052			H	0	1 0.332
				2 0.071					2 0.103
				3 0					3 0.105
			0.1	1 0.36				0.1	1 0.615
				2 0.24					2 0.567
				3 0.207					3 0.366
			0.2	1 0.496				0.2	1 5.136
				2 0.375					2 5.419
				3 0.815					3 5.115

Table B2. Raw data for the reoxidation of UO_2 in the presence of DFB and goethite at 3mM or 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)	U(VI) (μM)
2/16/10	0	3	0	1 0.09
				2 0.153
				3 0.081
			0.1	1 0.064
				2 0.121
				3 0.191
			0.2	1 0.048
				2 0.012
				3 0.025
		10	0	1 0.051
				2 0.13
				3 0.108
			0.1	1 0.114
				2 0.115
				3 0.156
			0.2	1 0.151
				2 0.123
				3 0.114
2/19/10	3	3	0	1 0.148
				2 0.096
				3 0.161
			0.1	1 0.154
				2 0.28
				3 0.261
			0.2	1 0.281
				2 0.366
				3 0.312
		10	0	1 0.475
				2 0.443
				3 0.375
			0.1	1 0.299
				2 0.366
				3 0.557
			0.2	1 0.585
				2 0.657
				3 0.227
2/23/10	7	3	0	1 0.276
				2 0.262
				3 0.181
			0.1	1 0.31
				2 0.391
				3 0.629
			0.2	1 0.551
				2 0.569
				3 0.767
		10	0	1 0.797
				2 0.314
				3 0.485
			0.1	1 0.547
				2 1.053
				3 1.264
			0.2	1 1.205
				2 0.116
				3 0.103
2/28/10	12	3	0	1 0.085
				2 0.103
				3 0.085

Table B2. Continued

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)		U(VI) (μ M)
3/5/10	17	10	0.1	1	0.31
				2	0.3
				3	0.284
			0.2	1	0.489
				2	0.565
				3	
			0	1	0.205
				2	0.82
				3	0.822
			0.1	1	0.597
				2	0.559
				3	0.568
			0.2	1	1.233
				2	1.427
				3	1.398
			0	1	0.149
				2	0.372
				3	0.233
			0.1	1	0.08
				2	0.3
				3	0.455
			0.2	1	0.369
				2	0.583
				3	
3/12/10	24	10	0	1	0.974
				2	1.386
				3	1.264
			0.1	1	0.569
				2	0.974
				3	1.055
			0.2	1	1.764
				2	1.887
				3	2.436
			0	1	0.245
				2	0.224
				3	0.253
			0.1	1	0.505
				2	0.588
				3	0.633
			0.2	1	0.83
				2	1.121
				3	
			0	1	1.608
				2	1.931
				3	1.767
			0.1	1	0.799
				2	1.045
				3	1.088
			0.2	1	1.755
				2	1.967
				3	2.081
3/23/10	35	3	0	1	0.253
				2	0.352
				3	0.346
			0.1	1	1.013
				2	1.117
				3	1.018
			0.2	1	1.993

Table B2. Continued

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)		U(VI) (μ M)
4/1/10	44	10	0	2	2.029
				3	
				1	3.143
			0.1	2	3.32
				3	3.054
				1	1.829
			0.2	2	1.96
				3	2.04
				1	2.111
		3	0	2	2.282
				3	2.617
				1	0.21
			0.1	2	0.229
				3	0.289
				1	0.847
			0.2	2	1.285
				3	1.048
				1	2.05
		10	0	2	2.271
				3	
				1	2.984
			0.1	2	4.052
				3	3.55
				1	1.682
			0.2	2	2.017
				3	1.896
				1	2.039
5/22/10	95	3	0	2	1.934
				3	1.976
				1	0
			0.1	2	0.239
				3	0.094
				1	1.492
			0.2	2	1.542
				3	1.522
				1	4.634
		10	0	2	5.227
				3	
				1	8.765
			0.1	2	10.138
				3	9.296
				1	4.177
			0.2	2	4.543
				3	4.487
				1	1.84
				2	2.054
				3	2.018

Table B3. Raw data for the reoxidation of UO_2 in the presence of DFB and hematite at 3mM or 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)		U(VI) (μM)
2/20/10	0	3	0	1	0.271
				2	0
				3	0
			0.1	1	0.271
				2	0.03
				3	0.124
			0.2	1	0.055
				2	0.363
				3	0.287
		10	0	1	0.004
				2	0.02
				3	0.147
			0.1	1	0.122
				2	0.369
				3	0.017
			0.2	1	0.157
				2	0.001
				3	0.036
2/24/10	4	3	0	1	0
				2	0
				3	0.005
			0.1	1	0.323
				2	0
				3	0.118
			0.2	1	0.716
				2	0.728
				3	0.772
		10	0	1	0.7
				2	0.667
				3	0.693
			0.1	1	0
				2	0
				3	0.216
			0.2	1	0.214
				2	0.46
				3	0.476
3/3/10	11	3	0	1	0.007
				2	0.048
				3	0.032
			0.1	1	0
				2	0
				3	0.083
			0.2	1	0.442
				2	0.53
				3	0.672
		10	0	1	1.017
				2	1.098
				3	1.016
			0.1	1	1.102
				2	0.721
				3	0.612
			0.2	1	0.687
				2	0.678
				3	0.874
3/11/10	19	3	0	1	0.194
				2	0.18
				3	0.203

Table B3. Continued

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)	U(VI) (μ M)
3/23/10	31	10	0.1	1 0.658
				2 0.472
				3 0.432
			0.2	1 0.532
				2 0.714
				3 0.862
			0	1 1.394
				2 2.048
				3 1.399
			0.1	1 1.005
				2 0.985
				3 0.055
			0.2	1 1.125
				2 1.227
				3 1.258
		3	0	1 0.242
				2 0.256
				3 0.27
			0.1	1 0.415
				2 0.509
				3 0.67
			0.2	1 1.627
				2 1.541
				3 1.594
			0	1 1.779
				2 1.793
				3 1.763
4/1/10	40	10	0.1	1 2.286
				2 2.564
				3 2.53
			0.2	1 2.408
				2 2.304
				3 2.504
			0	1 0.202
				2 0.19
				3 0.241
			0.1	1 0.593
				2 0.631
				3 0.743
			0.2	1 1.752
				2 1.885
				3 1.86
		3	0	1 1.693
				2 1.809
				3 1.813
			0.1	1 3.354
				2 3.433
				3 3.39
			0.2	1 2.549
				2 3.018
				3 4.055
5/25/10	94	3	0	1 0.035
				2 0.115
				3 0.009
			0.1	1 1.005
				2 0.932
				3 0.992
			0.2	1 2.526
				2 2.534

Table B3. Continued

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)	U(VI) (μ M)
		10	0	3 2.432
				1 1.83
				2 1.777
				3 1.777
			0.1	1 5.133
				2 4.896
				3 4.995
			0.2	1 9.293
				2 9.996
				3 10.432

Table B4. Raw data for the reoxidation of UO_2 in the presence of DFB and ferrihydrite at 3mM or 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)		U(VI) (μM)
2/22/10	0	3	0	1	0.802
				2	0.467
				3	0.353
			0.1	1	0.188
				2	0.158
				3	0.409
			0.2	1	0.117
				2	0.059
				3	0.106
		10	0	1	0.041
				2	0.097
				3	0.142
			0.1	1	0.083
				2	0.112
				3	0.087
			0.2	1	0.111
				2	0.13
				3	0.157
2/26/10	4	3	0	1	0.028
				2	0
				3	0
			0.1	1	0
				2	0
				3	0
			0.2	1	0
				2	0
				3	0
		10	0	1	0.883
				2	0.76
				3	0.813
			0.1	1	0.068
				2	0.116
				3	0.082
			0.2	1	0.059
				2	0.137
				3	0.201
3/5/10	11	3	0	1	0.401
				2	0.336
				3	0.38
			0.1	1	0.123
				2	0
				3	0
			0.2	1	0
				2	0
				3	0
		10	0	1	2.701
				2	2.206
				3	2.371
			0.1	1	1.441
				2	1.663
				3	1.751
			0.2	1	0.742
				2	
				3	0.324
3/12/10	18	3	0	1	0.529
				2	0.481
				3	0.52

Table B4. Continued

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)		U(VI) (μ M)
3/22/10	28	10	0.1	1	0.04
				2	0.068
				3	0.414
			0.2	1	0.083
				2	0.048
				3	0.044
			0	1	3.718
				2	4.387
				3	4.146
			0.1	1	3.527
				2	4.075
				3	3.433
			0.2	1	3.049
				2	
				3	2.952
			0	1	0.296
				2	0.556
				3	0.917
			0.1	1	0.173
				2	0.178
				3	2.849
			0.2	1	0.758
				2	0.962
				3	1.09
		10	0	1	9.569
				2	6.828
				3	6.826
			0.1	1	5.993
				2	5.96
				3	5.937
			0.2	1	6.149
				2	
				3	5.561
3/31/10	37	3	0	1	1.059
				2	1.285
				3	1.115
			0.1	1	0.041
				2	0.029
				3	0
			0.2	1	0.032
				2	0
				3	0
		10	0	1	9.142
				2	9.816
				3	9.683
			0.1	1	6.872
				2	6.735
				3	6.567
			0.2	1	6.186
				2	
				3	6.426
4/9/10	46	3	0	1	0.97
				2	0.922
				3	1.048
			0.1	1	0
				2	0
				3	0.222
			0.2	1	0

Table B4. Continued

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)	U(VI) (μM)
4/16/10	53	10	0	2
				3
				1
			0.1	2
				3
				1
			0.2	2
				3
				1
		3	0	2
				3
				1
			0.1	2
				3
				1
			0.2	2
				3
				1
		10	0	2
				3
				1
			0.1	2
				3
				1
			0.2	2
				3
				1
5/3/10	70	3	0	2
				3
				1
			0.1	2
				3
				1
			0.2	2
				3
				1
		10	0	2
				3
				1
			0.1	2
				3
				1
			0.2	2
				3
				1
6/6/10	104	3	0	2
				3
				1
			0.1	2
				3
				1
			0.2	2
				3
				1
		10	0	2
				3
				1
			0.1	2
				3
				1
			0.2	2
				3
				1

Table B4. Continued

Date	Days	Bicarbonate Conc. (mM)	DFB Conc. (mM)	U(VI) (μ M)
			3	8.967
			0.1	9.824
			2	9.101
			3	9.358
			0.2	8.918
			2	
			3	8.876

Table B5. Raw data for the reoxidation of UO₂ in the presence of FeCl₃ (0mM, 1.25mM, 2.5mM, or 25mM) showing U(VI) results from the KPA-11.

Date	Time	Hours		FeCl ₃ Conc. (mM)	KPA U(VI) (μM)	U(VI) Conc. (μM)
12/3/09	13:00	0	1	2.5	0.818	8.18
			2		0.729	7.29
			3		0.794	7.94
	13:15	0.25	1	2.5	54.903	549.03
			2		94.516	945.16
			3		88.528	885.28
	14:00	1	1	2.5	110.835	1108.35
			2		120.007	1200.07
			3		114.529	1145.29
	15:00	2	1	2.5	116.633	1166.33
			2		113.97	1139.7
			3		114.663	1146.63
12/4/09	9:45	20.75	1	2.5	114.233	1142.33
			2		117.971	1179.71
			3		117.179	1171.79
12/7/09	15:45	0	1	0	1.26	12.6
			2		1.87	18.7
			3		1.424	14.24
	16:00	0.25	1	0	4.05	40.5
			2		2.729	27.29
			3		3.585	35.85
	16:45	1	1	0	4.813	48.13
			2		4.822	48.22
			3		5.507	55.07
	10:45	19	1	0	7.186	71.86
			2		6.219	62.19
			3		8.546	85.46
12/9/09	9:45	0	1	1.25	1.049	10.49
			2		0.877	8.77
			3		0.788	7.88
	10:00	0.25	1	1.25	48.941	489.41
			2		48.408	484.08
			3		46.925	469.25
	10:45	1	1	1.25	49.136	491.36
			2		53.474	534.74
			3		51.772	517.72
	14:45	5	1	1.25	45.451	454.51
			2		51.589	515.89
			3		50.36	503.6
12/10/09	9:30	23.75	1	1.25	48.652	486.52
			2		51.085	510.85
			3		51.119	511.19
12/14/09	14:30	0	1	25	0.806	8.06
			2		0.81	8.1
			3		0.782	7.82
	14:45	0.25	1	25	116.097	1160.97
			2		123.833	1238.33
			3		123.095	1230.95
	15:30	1	1	25	147.644	1476.44
			2		153.496	1534.96
			3		153.841	1538.41
	16:30	2	1	25	139.897	1398.97
			2		142.791	1427.91
			3		147.086	1470.86
12/15/09	9:45	19.25	1	25	146.789	1467.89
			2		148.877	1488.77
			3		150.114	1501.14

Table B5. Continued

Date	Time	Hours		FeCl ₃ Conc. (mM)	KPA U(VI) (μM)	U(VI) Conc. (μM)
12/16/09	14:15	148.5	1	1.25	45.319	453.19
			2		49.996	499.96
			3		49.532	495.32

Table B6. Raw data for the reoxidation of UO_2 in the presence of 0.625mM DFB bound to 0.625mM Fe (1:1 complex) at pH 2.5 and 7 showing U(VI) results from the KPA-11.

Date	Time	Hours	pH		KPA U(VI) (μM)	U(VI) Conc. (μM)
2/22/10	14:45	0	2.5	1	0	0
				2	0	0
				3	0	0
			7	1	0	0
				2	0.116	1.16
				3	0.086	0.86
	15:00	0.25	2.5	1	0.426	4.26
				2	0.596	5.96
				3	1.198	11.98
			7	1	0	0
				2	0.004	0.04
				3	0	0
	16:00	1	2.5	1	1.89	18.9
				2	2.095	20.95
				3	2.184	21.84
			7	1	0.013	0.13
				2	0.932	9.32
				3	0	0
	18:00	3	2.5	1	2.818	28.18
				2	2.856	28.56
				3	2.981	29.81
			7	1	0.039	0.39
				2	0.038	0.38
				3	0.023	0.23
2/23/10	8:15	17.25	2.5	1	4.223	42.23
				2	4.713	47.13
				3	4.849	48.49
			7	1	0.169	1.69
				2	0.145	1.45
				3	1.752	17.52
2/24/10	14:00	35	2.5	1	5.179	51.79
				2	5.825	58.25
				3	5.849	58.49
			7	1	0.268	2.68
				2	0	0
				3	0.022	0.22
2/25/10	11:00	56	2.5	1	5.974	59.74
				2	6.602	66.02
				3	6.783	67.83
			7	1	0	0
				2	0	0
				3	0	0
2/26/10	12:45	81.75	2.5	1	6.3554	63.554
				2	6.86	68.6
				3	7.356	73.56
			7	1	0	0
				2	0	0
				3	0	0
2/28/10	16:15	109.25	2.5	1	7.498	74.98
				2	7.437	74.37
				3	8.081	80.81
			7	1	0	0
				2	0	0
				3	0	0
3/3/10	15:15	180.25	2.5	1	7.497	74.97
				2	8.832	88.32
				3	8.686	86.86

Table B6. Continued

Date	Time	Hours	pH		KPA U(VI) (μ M)	U(VI) Conc. (μ M)
3/10/10	11:00	344	7	1	0	0
				2	0	0
				3	0	0
			2.5	1	8.712	87.12
				2	9.216	92.16
				3	8.664	86.64
			7	1	0	0
				2	0	0
				3	0	0

Table B7. Raw data for the reoxidation of UO_2 in the presence of 1.25mM DFB bound to 1.25mM Fe (1:1 complex) at pH 2.4 and 7 showing U(VI) results from the KPA-11.

Date	Time	Hours	pH		KPA U(VI) (μM)	U(VI) Conc. (μM)
3/2/10	15:30	0	2.4	1	0	0
				2	0	0
				3	0	0
			7	1	0	0
				2	0	0
				3	0	0
	15:45	0.25	2.4	1	0.439	4.39
				2	0.685	6.85
				3	0.744	7.44
			7	1	0	0
				2	0	0
				3	0	0
	16:45	1	2.4	1	2.141	21.41
				2	3.417	34.17
				3	2.441	24.41
			7	1	0	0
				2	0	0
				3	0	0
3/3/10	10:15	18.5	2.4	1	4.061	40.61
				2	4.416	44.16
				3	4.86	48.6
			7	1	0	0
				2	0	0
				3	0.398	3.98
	16:15	24.5	2.4	1	4.438	44.38
				2	4.721	47.21
				3	4.975	49.75
			7	1	0	0
				2	0	0
				3	0.107	1.07
3/4/10	14:00	46.25	2.4	1	4.808	48.08
				2	5.488	54.88
				3	5.866	58.66
			7	1	0	0
				2	0.252	2.52
				3	0.556	5.56
3/5/10	14:30	70.75	2.4	1	6.076	60.76
				2	6.314	63.14
				3	6.732	67.32
			7	1	0	0
				2	0	0
				3	0.277	2.77
3/8/10	12:15	140.5	2.4	1	6.684	66.84
				2	7.244	72.44
				3	7.784	77.84
			7	1	0	0
				2	0.082	0.82
				3	0.049	0.49

Table B8. Raw data for the reoxidation of UO_2 in the presence of 2.5mM ferric citrate at pH 2.58, 5.4, and 7.1 showing U(VI) results from the KPA-11.

Date	Time	Hours	pH		KPA U(VI) (μM)	U(VI) Conc. (μM)
3/4/10	10:00	0	2.58	1	0	0
				2	0	0
				3	0	0
			5.4	1	0	0
				2	0	0
				3	0	0
			7.1	1	0	0
				2	0	0
				3	0	0
	10:15	0.25	2.58	1	9.036	90.36
				2	15.286	152.86
				3	17.506	175.06
			5.4	1	0.072	0.72
				2	0.215	2.15
				3	0.045	0.45
			7.1	1	0.091	0.91
				2	0.197	1.97
				3	0.141	1.41
	11:00	0.75	2.58	1	51.783	517.83
				2	52.264	522.64
				3	52.092	520.92
	12:15	1.25	5.4	1	0.793	7.93
				2	0.935	9.35
				3	1.536	15.36
		0.75	7.1	1	0.457	4.57
				2	0.512	5.12
				3	0.595	5.95
	21:00	10.75	2.58	1	58.79	587.9
				2	57.861	578.61
				3	57.494	574.94
		10	5.4	1	2.143	21.43
				2	2.065	20.65
				3	2.153	21.53
		10.75	7.1	1	1.045	10.45
				2	1.257	12.57
				3	1.599	15.99
3/5/10	14:30	28.25	2.58	1	63.243	632.43
				2	65.816	658.16
				3	65.542	655.42
		27.5	5.4	1	3.29	32.9
				2	3.324	33.24
				3	3.307	33.07
		28.25	7.1	1	1.701	17.01
				2	1.928	19.28
				3	2.015	20.15
3/8/10	11:45	97.5	2.58	1	60.875	608.75
				2	64.406	644.06
				3	64.914	649.14
		97.25	5.4	1	5.242	52.42
				2	4.394	43.94
				3	4.556	45.56
		97.5	7.1	1	2.247	22.47
				2	2.703	27.03
				3	2.485	24.85
3/12/10	12:00	193.75	2.58	1	65.963	659.63
				2	65.555	655.55
				3	68.309	683.09

Table B8. Continued

Date	Time	Hours	pH		KPA U(VI) (μM)	U(VI) Conc. (μM)
4/23/10	14:00	193.5	5.4	1	3.749	37.49
				2	4.22	42.2
				3	4.378	43.78
		193.75	7.1	1	2.723	27.23
				2	1.856	18.56
				3	3.05	30.5
			2.58	1	51.829	518.29
				2	55.104	551.04
				3	54.191	541.91
			5.4	1	1.898	18.98
				2	2.358	23.58
				3	3.367	33.67
			7.1	1	1.204	12.04
				2	0.564	5.64
				3	0.106	1.06

Table B9. Raw data for the reoxidation of UO_2 in the presence of citrate and ferrihydrite with 0mM, 3mM, or 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μM)	Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μM)
3/30/10	0	0	0	1	5/11/10	42	0	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
	3	0	0	1		42	0	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
	10	0	0	1		42	0	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
4/5/10	6	0	0	1	5/18/10	49	0	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
	3	0	0	1		49	0	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
	10	0	0	1		49	0	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3

Table B9. Continued

Date	Days	Bicarb Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)	Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)
4/10/10	11	0	0	2 3.161	5/28/10	59	0	0	2 0.612
				3 5.078					3 0.767
				0.1 1 7.467					0.1 1 2.141
				2 7.204					2 2.346
				3 9.769					3 2.755
				0.2 1 10.772					0.2 1 12.039
				2 10.378					2 13.076
				3 12.017					3 11.827
				1 0.134					1 0.525
				2 0					2 0.503
				3 0					3 0.553
				0.1 1 0.016					0.1 1 1.931
				2 0.058					2 1.978
				3 0.085					3 2.01
				0.2 1 1.691					0.2 1 11.513
				2 1.934					2 11.952
				3 1.801					3 12.031
		3	0	1 0.351			3	0	1 0.576
				2 0.358					2 0.605
				3 0.277					3 0.612
				0.1 1 0.845					0.1 1 1.86
				2 1.176					2 2.058
				3 1.412					3 1.822
				0.2 1 6.372					0.2 1 10.253
				2 6.651					2 11.12
				3 6.878					3 10.885
		10	0	1 1.067			10	0	1 0.612
				2 0.741					2 0.985
				3 1.412					3 0.823
				0.1 1 3.248					0.1 1 2.057
				2 3.375					2 2.043
				3 3.575					3 2.71
				0.2 1 11.667					0.2 1 14.273
				2 12.941					2 14.341
				3 14.47					3 14.382
4/19/10	20	0	0	1 0.401	6/7/10	69	0	0	1 0.472
				2 0.607					2 0.472
				3 0.184					3 0.527
				0.1 1 0.347					0.1 1 1.999
				2 0.376					2 2.032
				3 0.38					3 3.714
				0.2 1 3.801					0.2 1 12.884
				2 4.207					2 13.249
				3 4.789					3 14.67
		3	0	1 0.494			3	0	1 0.53
				2 0.477					2 0.569
				3 0.433					3 0.592

Table B9. Continued

Date	Days	Bicarb Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)	Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)
4/27/10	28	0	0.1	1	6/21/10	83	0	0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
		10	0	1			10	0	1
				2					2
				3					3
		0.1		1				0.1	1
				2					2
				3					3
		0.2		1				0.2	1
				2					2
				3					3
		3	0	1			3	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
		10	0	1			10	0	1
				2					2
				3					3
			0.1	1				0.1	1
				2					2
				3					3
			0.2	1				0.2	1
				2					2
				3					3
5/4/10	35	0	0	1					1
				2					2
				3					3
				1					1
				2					2
				3					3
				1					1
				2					2

Table B9. Continued

Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)	Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)
				3 0.951					
			0.2	1 8.21					
				2 8.137					
				3 7.925					
		3	0	1 0.476					
				2 0.498					
				3 0.487					
			0.1	1 1.447					
				2 1.585					
				3 1.628					
			0.2	1 8.504					
				2 9.114					
				3 8.763					
		10	0	1 0.605					
				2 0.609					
				3 0.779					
			0.1	1 2.39					
				2 2.266					
				3 2.821					
			0.2	1 12.027					
				2 12.103					
				3 11.791					

Table B10. Raw data for the reoxidation of UO_2 in the presence of citrate and hematite with 0mM, 3mM, or 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Bicar Conc. (mM)	Citr. Conc. (mM)		U(VI) (μM)	Date	Days	Bicarb. Conc. (mM)	Citr. Conc. (mM)		U(VI) (μM)
4/8/10	0	0	0	1	0.006	5/14/10	36	0	0	1	0.217
				2	0					2	0.047
				3	0					3	0
			0.1	1	0				0.1	1	0
				2	0					2	0.155
				3	0					3	0.495
			0.2	1	0				0.2	1	0.262
				2	0					2	0
				3	0					3	0
		3	0	1	0.209			3	0	1	0.067
				2	0					2	0.057
				3	0					3	0.051
			0.1	1	0				0.1	1	0.093
				2	0					2	0.053
				3	0					3	0.047
			0.2	1	0.005				0.2	1	0.019
				2	0					2	0.016
				3	0					3	0.262
		10	0	1	0			10	0	1	0.886
				2	0					2	1.102
				3	0					3	1.205
			0.1	1	0				0.1	1	1.158
				2	0					2	1.088
				3	0.077					3	1.204
			0.2	1	0				0.2	1	1.011
				2	0					2	0.898
				3	0					3	0.844
4/15/10	7	0	0	1	0	5/21/10	43	0	0	1	0
				2	0.005					2	0
				3	0					3	0
			0.1	1	0				0.1	1	0
				2	0.129					2	0
				3	0.269					3	0
			0.2	1	0.858				0.2	1	0.032
				2	0.509					2	0
				3	0.451					3	0
		3	0	1	0			3	0	1	0
				2	0					2	0.031
				3	0					3	0.771
			0.1	1	0.315				0.1	1	0.28
				2	0.394					2	0.351
				3	0.516					3	0.019
			0.2	1	0.747				0.2	1	0.039
				2	0.655					2	0.053
				3	0.748					3	0.037
		10	0	1	0.381			10	0	1	1.002
				2	0.565					2	1.215
				3	0.558					3	1.197
			0.1	1	0.431				0.1	1	0.851
				2	0.569					2	1.016
				3	0.831					3	1.267
			0.2	1	0.774				0.2	1	0.829
				2	0.699					2	0.839
				3	0.629					3	0.783
4/23/10	15	0	0	1	0.029	6/4/10	57	0	0	1	0
				2	0					2	0.023

Table B10. Continued

Date	Days	Bicar. Conc. (mM)	Citr. Conc. (mM)	U(VI) (μ M)	Date	Days	Bicarb. Conc. (mM)	Citr. Conc. (mM)	U(VI) (μ M)
4/30/10	22	0	0	3 0.038	6/20/10	73	0	0	3 0
				0.1 1 0.044					0.1 1 0
				2 0.054					2 0
				3 0.093					3 0
				0.2 1 0.057					0.2 1 0
				2 0.015					2 0
				3 0.023					3 0
				3 0 1 0.053					3 0 1 0.031
				2 0.411					2 0.007
				3 0.322					3 0.005
				0.1 1 0.084					0.1 1 0.022
				2 0.147					2 0
				3 0.106					3 0.01
				0.2 1 0.145					0.2 1 0
				2 0.12					2 0.027
				3 0.184					3 0.073
				10 0 1 0.786					10 0 1 1.093
				2 0.854					2 1.244
				3 0.847					3 1.224
				0.1 1 0.798					0.1 1 0.833
				2 0.909					2 1.118
				3 1.115					3 1.124
				0.2 1 1.099					0.2 1 0.792
				2 1.457					2 0.798
				3 0.963					3 1.005
				1 0					1 0
				2 0					2 0
				3 0					3 0
				0.1 1 0.211					0.1 1 0
				2 0.004					2 0
				3 0.186					3 0
				0.2 1 0					0.2 1 0
				2 0					2 0
				3 0.071					3 0
				3 0 1 0.031					3 0 1 0.033
				2 0.012					2 0.041
				3 0.117					3 0.049
				0.1 1 0.039					0.1 1 0.132
				2 0.073					2 0.101
				3 0.072					3 0
				0.2 1 0.084					0.2 1 0
				2 0.091					2 0
				3 0.108					3 0
				10 0 1 1.024					10 0 1 0.957
				2 1.005					2 2.943
				3 0.944					3 3.763
				0.1 1 1.038					0.1 1 0.768
				2 0.991					2 0.826
				3 1.164					3 0.87
				0.2 1 1.081					0.2 1 0.759
				2 1.096					2 0.58
				3 1.008					3 0.499
5/7/10	29	0	0	1 0	6/30/10	83	0	0	1 0
				2 0					2 0
				3 0					3 0
				0.1 1 0					0.1 1 0

Table B10. Continued

Date	Days	Bicar Conc. (mM)	Citr. Conc. (mM)	U(VI) (μ M)	Date	Days	Bicarb. Conc. (mM)	Citr. Conc. (mM)	U(VI) (μ M)
			2	0				2	0
			3	0.021				3	0
		0.2	1	0				0.2	1
			2	0				2	0
			3	0				3	0
	3	0	1	0.171		3	0	1	0.1
			2	0				2	0
			3	0.055				3	0
		0.1	1	0				0.1	1
			2	0.013				2	0
			3	0				3	0.103
		0.2	1	0				0.2	1
			2	0.067				2	0.118
			3	0.023				3	0
	10	0	1	0.859		10	0	1	0.936
			2	0.934				2	0.963
			3	0.93				3	0.994
		0.1	1	0.819				0.1	1
			2	0.959				2	0.935
			3	1.083				3	0.944
		0.2	1	0.841				0.2	1
			2	0.847				2	0.533
			3	0.803				3	0.534

Table B11. Raw data for the reoxidation of UO_2 in the presence of citrate and goethite with 0mM, 3mM, or 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Bicar Conc. (mM)	Citr. Conc. (mM)	U(VI) (μM)	Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μM)
5/1/10	0	0	0	1 0.009	6/12/10	42	0	0	1 0
				2 0					2 0
				3 0					3 0
			0.1	1 0.006				0.1	1 0
				2 0					2 0
				3 0					3 0
			0.2	1 0.134				0.2	1 0
				2 0.047					2 0
				3 0.073					3 0
		3	0	1 0			3	0	1 0.055
				2 0					2 0
				3 0					3 0
			0.1	1 0.209				0.1	1 0
				2 0					2 0
				3 0					3 0.249
			0.2	1 0.019				0.2	1 0
				2 0.013					2 0
				3 0					3 0
		10	0	1 0.23			10	0	1 1.28
				2 0.038					2 1.413
				3 0.003					3 1.554
			0.1	1 0				0.1	1 1.257
				2 1.1					2 1.449
				3 0					3 1.432
			0.2	1 0.07				0.2	1 0.979
				2 0					2 1.02
				3 0.165					3 0.633
5/8/10	7	0	0	1 0	6/23/10	53	0	0	1 0
				2 0					2 0
				3 0					3 0
				0.1 1 0.04					0.1 1 0
				2 0					2 0
				3 0.056					3 0
				0.2 1 0.695					0.2 1 0
				2 0.727					2 0
				3 0.535					3 0
		3	0	1 0			3	0	1 0
				2 0					2 0
				3 0.012					3 0
			0.1	1 0				0.1	1 0
				2 0.013					2 0
				3 0					3 0
			0.2	1 0				0.2	1 0
				2 0.028					2 0
				3 0.205					3 0
		10	0	1 0.424			10	0	1 1.333
				2 0.548					2 1.695
				3 0.618					3 1.442
			0.1	1 0.475				0.1	1 1.305
				2 0.502					2 1.372
				3 0.538					3 1.365
			0.2	1 0.403				0.2	1 0.795
				2 0.469					2 0.907
				3 0.552					3 0.477
5/16/10	15	0	0	1 0	7/6/10	66	0	0	1 0
				2 0.076					2 0

Table B11. Continued

Date	Days	Bicar. Conc. (mM)	Citr. Conc. (mM)	U(VI) (μ M)	Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)
5/24/10	23	0	0	3 0	7/20/10	80	0	0	3 0
				0.1 1 0.026					0.1 1 0.112
				2 0.029					2 0
				3 0.081					3 0
				0.2 1 0.042					0.2 1 0
				2 0					2 0
				3 0					3 0
				3 0 1 0.014					3 0 1 0
				2 0.051					2 0
				3 0.167					3 0
				0.1 1 0.144					0.1 1 0
				2 0.113					2 0
				3 0.011					3 0.151
				0.2 1 0.023					0.2 1 0
				2 0.01					2 0
				3 0					3 0
				10 0 1 1					10 0 1 1.54
				2 1.206					2 1.657
				3 1.332					3 1.788
				0.1 1 1.109					0.1 1 1.454
				2 1.109					2 1.681
				3 1.325					3 1.744
				0.2 1 1.074					0.2 1 0.971
				2 1.085					2 0.994
				3 0.906					3 0.698
				1 0					1 0.34
				2 0					2 0
				3 0					3 0
				0.1 1 0					0.1 1 0
				2 0					2 0
				3 0					3 0
				0.2 1 0					0.2 1 0
				2 0.001					2 0
				3 0.238					3 0
				3 0 1 0					3 0 1 0
				2 0					2 0
				3 0					3 0
				0.1 1 0.134					0.1 1 0
				2 0.159					2 0
				3 0.124					3 0
				0.2 1 0					0.2 1 0
				2 0					2 0
				3 0.183					3 0
				10 0 1 1.216					10 0 1 1.608
				2 1.336					2 1.59
				3 1.407					3 1.608
				0.1 1 1.304					0.1 1 1.08
				2 1.427					2 1.494
				3 1.469					3 1.302
				0.2 1 1.507					0.2 1 0.701
				2 1.121					2 0.697
				3 0.887					3 0.286
6/3/10	33	0	0	1 0					
				2 0					
				3 0					
				0.1 1 0					

Table B11. Continued

Date	Days	Bicar Conc. (mM)	Citr. Conc. (mM)	U(VI) (μ M)	Date	Days	Bicarb. Conc. (mM)	Citrate Conc. (mM)	U(VI) (μ M)
				2					0
				3					0
			0.2	1					0
				2					0.381
				3					0.194
	3	0		1					0.255
				2					0.257
				3					0.276
		0.1		1					0.343
				2					0.392
				3					0.085
		0.2		1					0.008
				2					0.028
				3					0.025
	10	0		1					1.503
				2					1.552
				3					1.658
		0.1		1					1.45
				2					1.637
				3					1.703
		0.2		1					1.172
				2					1.259
				3					0.872

Table B12. Raw data for the reoxidation of UO_2 in the presence of EDTA and ferrihydrite (F), goethite (G), or hematite (H) with 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Iron oxide	EDTA Conc. (mM)	U(VI) (μM)	Date	Days	Iron oxide	EDTA Conc. (mM)	U(VI) (μM)
6/8/10	0	F	0	1 0.087	7/23/10	45	F	0	1 5.4
				2 0.007					2 3.964
				3 0					3 4.98
			0.1	1 0.014				0.1	1 43.775
				2 0.019					2 43.227
				3 0					3 45.436
			0.2	1 0.001				0.2	1 68.582
				2 0					2 72.854
				3 0.059					3 70.186
		G	0	1 0.114			G	0	1 0.948
				2 0.142					2 1.155
				3 0.158					3 2.466
			0.1	1 0.174				0.1	1 22.121
				2 0.292					2 24.251
				3 0.246					3 23.696
			0.2	1 0.167				0.2	1 66.492
				2 0.076					2 69.033
				3 0.025					3 67.895
		H	0	1 0.005			H	0	1 0.587
				2 0.023					2 0.624
				3 0.036					3 0.777
			0.1	1 0				0.1	1 27.264
				2 0.014					2 28.921
				3 0					3 23.861
			0.2	1 0.155				0.2	1 71.202
				2 0					2 72.491
				3 0.004					3 74.981
6/14/10	6	F	0	1 0.273	8/2/10	55	F	0	1 6.632
				2 0.277					2 4.927
				3 0.256					3 6.176
			0.1	1 31.675				0.1	1 44.373
				2 29.26					2 44.509
				3 29.471					3 46.428
			0.2	1 52.568				0.2	1 71.593
				2 59.581					2 74.44
				3 59.551					3 71.838
		G	0	1 0.166			G	0	1 1.055
				2 0.335					2 1.134
				3 0.366					3 0.949
			0.1	1 25.624				0.1	1 21.708
				2 28.727					2 24.709
				3 27.333					3 24.439
			0.2	1 55.976				0.2	1 66.822
				2 48.129					2 66.154
				3 50.284					3 66.683
		H	0	1 0.204			H	0	1 0.686
				2 0.331					2 0.757
				3 0.208					3 0.709
			0.1	1 29.074				0.1	1 26.955
				2 29.097					2 27.226
				3 27.698					3 23.912
			0.2	1 40.93				0.2	1 68.106
				2 56.263					2 73.1
				3 61.469					3 74.097

Table B12. Continued

Date	Days	Iron oxide	EDTA Conc. (mM)		U(VI) (μ M)	Date	Days	Iron oxide	EDTA Conc. (mM)	U(VI) (μ M)
6/21/10	13	F	0	1	1.778					
				2	0.755					
				3	0.836					
			0.1	1	34.315					
				2	33.344					
				3	32.599					
			0.2	1	55.916					
				2	58.756					
				3	59.956					
		G	0	1	0.793					
				2	0.996					
				3	0.751					
			0.1	1	23.682					
				2	26.614					
				3	25.566					
			0.2	1	65.911					
				2	67.766					
				3	67.125					
		H	0	1	0.609					
				2	0.548					
				3	0.832					
			0.1	1	28.638					
				2	29.312					
				3	26.696					
			0.2	1	68.757					
				2	74.974					
				3	78.791					
7/1/10	23	F	0	1	1.345					
				2	1.016					
				3	1.323					
			0.1	1	41.382					
				2	39.05					
				3	41.447					
			0.2	1	62.036					
				2	64.553					
				3	62.677					
		G	0	1	0.787					
				2	1.009					
				3	0.876					
			0.1	1	23.445					
				2	25.994					
				3	24.861					
			0.2	1	66.16					
				2	68.882					
				3	67.728					
		H	0	1	0.459					
				2	0.568					
				3	0.695					
			0.1	1	28.196					
				2	29.605					
				3	26.629					
			0.2	1	68.741					
				2	72.409					
				3	75.917					
7/13/10	35	F	0	1	3.105					
				2	2.177					
				3	2.755					

Table B12. Continued

Date	Days	Iron oxide	EDTA Conc. (mM)	U(VI) (μ M)	Date	Days	Iron oxide	EDTA Conc. (mM)	U(VI) (μ M)
			0.1	1 42.355					
				2 41.292					
				3 41.8					
			0.2	1 61.512					
				2 64.512					
				3 62.111					
		G	0	1 0.985					
				2 1.074					
				3 0.836					
			0.1	1 22.778					
				2 24.084					
				3 24.086					
			0.2	1 65.833					
				2 62.653					
				3 63.698					
		H	0	1 0.541					
				2 0.603					
				3 0.718					
			0.1	1 25.854					
				2 26.405					
				3 22.834					
			0.2	1 63.519					
				2 67.077					
				3 70.669					

Table B13. Raw data for the reoxidation of UO_2 in the presence of chelators (0.2mM) and ferrihydrite with 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Chelator		U(VI) (μM)	Date	Days	Chelator		U(VI) (μM)
5/9/10	0	Acetate	1	0	5/17/10	0	Citrate	1	0
			2	0				2	0
			3	0				3	0.011
		Glucose	1	0			EDTA	1	0
			2	0.125				2	0.011
			3	0				3	0
		Pyruvate	1	0			NTA	1	0.041
			2	0.04				2	0
			3	0				3	0
		Malate	1	0	5/24/10	7	Citrate	1	8.933
			2	0.177				2	10.911
			3	0.254				3	9.907
		Succinate	1	0.746			EDTA	1	62.865
			2	0.375				2	62.317
			3	0.149				3	61.19
5/16/10	7	Acetate	1	0.864			NTA	1	54.95
			2	0.467				2	55.897
			3	0.495				3	54.366
		Glucose	1	0.455	6/1/10	15	Citrate	1	9.029
			2	0.417				2	10.666
			3	0.47				3	9.029
		Pyruvate	1	0.501			EDTA	1	63.642
			2	0.469				2	61.482
			3	0.448				3	65.435
		Malate	1	1.865			NTA	1	56.483
			2	1.571				2	56.44
			3	1.247				3	57.605
		Succinate	1	0.276	6/11/10	25	Citrate	1	9.574
			2	0.482				2	11.201
			3	0.613				3	10.49
5/24/10	15	Acetate	1	3.072			EDTA	1	64.47
			2	2.223				2	66.054
			3	1.703				3	63.781
		Glucose	1	1.439			NTA	1	59.497
			2	1.683				2	58.113
			3	1.301				3	58.111
		Pyruvate	1	1.459	6/21/10	35	Citrate	1	11.67
			2	1.411				2	13.345
			3	1.465				3	11.912
		Malate	1	4.844			EDTA	1	73.032
			2	4.233				2	72.382
			3	3.717				3	73.101
		Succinate	1	1.197			NTA	1	64.519
			2	1.406				2	63.839
			3	1.466				3	63.567
6/3/10	25	Acetate	1	5.231	7/1/10	45	Citrate	1	10.005
			2	3.796				2	14.631
			3	3.495				3	10.982
		Glucose	1	3.428			EDTA	1	72.933
			2	3.415				2	71.515
			3	3.274				3	74.569
		Pyruvate	1	3.381			NTA	1	63.822
			2	3.594				2	64.459
			3	3.368				3	64.326
		Malate	1	7.169	7/14/10	58	Citrate	1	10.256
			2	6.427				2	13.255
			3	5.617				3	10.96

Table B13. Continued

Date	Days	Chelator		U(VI) (μ M)	Date	Days	Chelator		U(VI) (μ M)
6/13/10	35	Succinate	1	3.149	7/28/10	72	EDTA	1	69.973
			2	3.113				2	69.003
			3	3.566				3	70.48
		Acetate	1	7.346			NTA	1	60.953
			2	6.435				2	61.55
			3	6.259				3	62.585
		Glucose	1	5.345			Citrate	1	11.342
			2	5.588				2	15.08
			3	5.291				3	13.245
		Pyruvate	1	6.165			EDTA	1	78.017
			2	5.79				2	75.433
			3	5.693				3	80.207
		Malate	1	8.45			NTA	1	65.329
			2	8.001				2	69.353
			3	6.361				3	70.524
6/23/10	45	Succinate	1	5.409					
			2	5.899					
			3	6.579					
		Acetate	1	9.212					
			2	8.307					
			3	8.004					
		Glucose	1	6.428					
			2	6.335					
			3	6.308					
		Pyruvate	1	7.371					
			2	7.559					
			3	7.483					
		Malate	1	9.612					
			2	8.883					
			3	6.98					
7/6/10	58	Succinate	1	6.543					
			2	7.604					
			3	8.15					
		Acetate	1	13.872					
			2	11.691					
			3	11.231					
		Glucose	1	7.841					
			2	7.316					
			3	7.43					
		Pyruvate	1	9.676					
			2	9.639					
			3	9.56					
		Malate	1	10.811					
			2	10.189					
			3	8.682					
7/20/10	72	Succinate	1	7.951					
			2	9.768					
			3	9.972					
		Acetate	1	14.462					
			2	11.54					
			3	11.356					
		Glucose	1	6.954					
			2	6.701					
			3	6.703					
		Pyruvate	1	10.391					
			2	10.929					
			3	10.446					
		Malate	1	11.375					

Table B13. Continued

Date	Days	Chelator	U(VI) (μM)	Date	Days	Chelator	U(VI) (μM)
			2				11.145
			3				9.885
		Succinate	1				8.392
			2				10.434
			3				11.056

Table B14. Raw data for the reoxidation of UO_2 in the presence of NTA and ferrihydrite (F), goethite (G), or hematite (H) with 10mM bicarbonate showing U(VI) results from the KPA-11.

Date	Days	Iron oxide	NTA Conc. (mM)	U(VI) (uM)	Date	Days	Iron oxide	NTA Conc. (mM)	U(VI) (uM)
6/8/10	0	F	0	1 0.09	7/23/10	45	F	0	1 5.4
				2 0.01					2 3.96
				3 0					3 4.98
			0.1	1 0.03				0.1	1 30.1
				2 0.11					2 31.1
				3 0.1					3 32.2
			0.2	1 0				0.2	1 45.7
				2 0					2 46.7
				3 0.18					3 44.9
		G	0	1 0.11			G	0	1 0.95
				2 0.14					2 1.16
				3 0.16					3 2.47
			0.1	1 0				0.1	1 5.3
				2 0.11					2 5.19
				3 0.02					3 5.31
			0.2	1 0				0.2	1 9.57
				2 0.03					2 9.93
				3 0.01					3 8.42
		H	0	1 0.01			H	0	1 0.59
				2 0.02					2 0.62
				3 0.04					3 0.78
			0.1	1 0				0.1	1 7.41
				2 0.07					2 4.85
				3 0.42					3 6.31
			0.2	1 0.02				0.2	1 7.88
				2 0					2 13.9
				3 0					3 13.5
6/14/10	6	F	0	1 0.27	8/2/10	55	F	0	1 6.63
				2 0.28					2 4.93
				3 0.26					3 6.18
			0.1	1 15.7				0.1	1 29.7
				2 16.7					2 32.7
				3 17.3					3 33.1
			0.2	1 29.9				0.2	1 47.7
				2 28.8					2 48.7
				3 28.2					3 48.2
		G	0	1 0.17			G	0	1 1.06
				2 0.34					2 1.13
				3 0.37					3 0.95
			0.1	1 3.84				0.1	1 5.48
				2 3.33					2 5.26
				3 3.42					3 5.2
			0.2	1 6.9				0.2	1 9.42
				2 6.51					2 10.2
				3 5.74					3 8.69
		H	0	1 0.2			H	0	1 0.69
				2 0.33					2 0.76
				3 0.21					3 0.71
			0.1	1 3.95				0.1	1 7.55
				2 3.62					2 5.02
				3 3.64					3 6.32
			0.2	1 5.31				0.2	1 8.37
				2 6.77					2 13.7
				3 6.63					3 14.1
6/21/10	13	F	0	1 1.78					

Table B14. Continued

Date	Days	Iron oxide	NTA Conc. (mM)	U(VI) (uM)	Date	Days	Iron oxide	NTA Conc. (mM)	U(VI) (uM)
7/1/10	23	G		2	0.76				
				3	0.84				
			0.1	1	21				
				2	20.7				
				3	21.1				
			0.2	1	31				
				2	32.7				
				3	32.8				
			0	1	0.79				
				2	1				
				3	0.75				
			0.1	1	5.8				
				2	5.83				
				3	5.43				
			0.2	1	8.79				
				2	9.26				
				3	8.03				
		H	0	1	0.61				
				2	0.55				
				3	0.83				
			0.1	1	7.12				
				2	5.27				
				3	5.86				
			0.2	1	7.79				
				2	9.69				
				3	10.5				
		F	0	1	1.35				
				2	1.02				
				3	1.32				
			0.1	1	28.9				
				2	29.1				
				3	29.9				
			0.2	1	38.1				
				2	40.1				
				3	40.3				
		G	0	1	0.79				
				2	1.01				
				3	0.88				
			0.1	1	5.28				
				2	5.35				
				3	5.03				
			0.2	1	8.46				
				2	9.13				
				3	7.87				
		H	0	1	0.46				
				2	0.57				
				3	0.7				
			0.1	1	7.21				
				2	4.92				
				3	5.94				
			0.2	1	8.01				
				2	10.5				
				3	11.4				
7/13/10	35	F	0	1	3.11				
				2	2.18				
				3	2.76				
			0.1	1	28.9				

Table B14. Continued

Date	Days	Iron oxide	NTA Conc. (mM)	U(VI) (uM)	Date	Days	Iron oxide	NTA Conc. (mM)	U(VI) (uM)
				2	29.2				
				3	29.6				
			0.2	1	40.8				
				2	41.2				
				3	42.2				
		G	0	1	0.99				
				2	1.07				
				3	0.84				
			0.1	1	5				
				2	5.01				
				3	4.83				
			0.2	1	8.43				
				2	8.84				
				3	7.38				
		H	0	1	0.54				
				2	0.6				
				3	0.72				
			0.1	1	6.85				
				2	4.75				
				3	5.55				
			0.2	1	7.25				
				2	10.1				
				3	11.1				

APPENDIX C

RAW DATA FOR Fe(III) (HYDR)OXIDE DISSOLUTION EXPERIMENTS

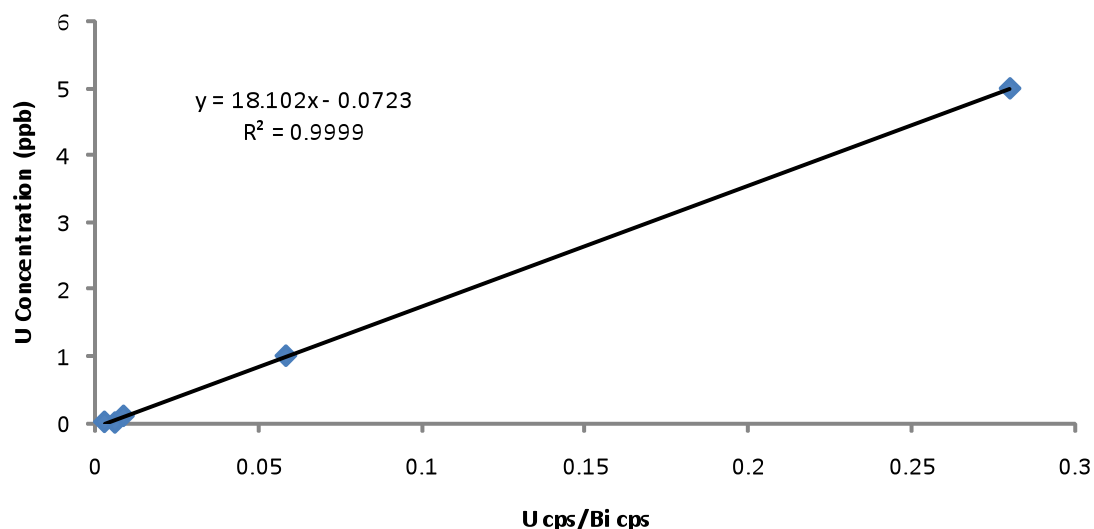


Figure C1. A uranium calibration curve from ICP-MS data for measuring total aqueous uranium from UO_2 dissolution experiments. All calibration curves for U were set up like this and standards were ran every time samples were being analyzed.

Table C1. Raw ICP-MS data for total aqueous uranium from UO_2 dissolution with DFB (0mM, 0.1mM, or 0.2mM) with 0mM bicarbonate after 20 days.

Sample ID	Uranium / 238 [#3]	Bismuth / 209 [#3]	U cps/Bi cps	U Conc. (ppb)	U Conc. in samples (ppb)	U Conc. in samples (μM)
0	3017.13	473885	0.0064	0.043		
0.01	1510.14	479209	0.0032	-0.015		
0.1	4406.46	489713	0.009	0.0906		
1	29762.76	507083	0.0587	0.9902		
5	132711.5	473470	0.2803	5.0016		
10	264070.3	478715	0.5516	9.9132		
50	2037537	488573	4.1704	75.42		
100	3937771	485250	8.1149	146.82		
500	18771780	493395	38.046	688.64		
0	4031.9	465393	0.0087	0.0845		
0	8031.66	457861	0.0175	0.2452		
0	5879.34	459315	0.0128	0.1594		
10ppb U- PIPES	247187.4	460503	0.5368	9.6444		
50ppb U- PIPES	1947522	466650	4.1734	75.475		
0DFB 1	44516.25	453613	0.0981	1.7042	170.4	0.71595
0DFB 2	55985.01	450215	0.1244	2.1787	217.9	0.91531
0DFB 3	42038.18	452430	0.0929	1.6097	161	0.67625
0.1DFB 1	101870.3	455078	0.2239	3.9799	398	1.67201
0.1DFB 2	99654.46	452408	0.2203	3.9151	391.5	1.64481
0.1DFB 3	102500.8	450720	0.2274	4.0444	404.4	1.69911
0.2DFB 1	124163.1	452618	0.2743	4.8935	489.3	2.05583
0.2DFB 2	139067.3	452241	0.3075	5.4942	549.4	2.30819
0.2DFB 3	137546.6	444175	0.3097	5.5333	553.3	2.32462

Table C2. Raw ICP-MS data for total aqueous uranium from UO₂ dissolution with DFB (0mM, 0.1mM, or 0.2mM) with 3mM or 10mM bicarbonate after 6 days.

Sample	Bi #2	U #2	U/Bi #2	U Conc. (ppb)	U Conc.(ppb in Samp)	U Conc. in Samp(μM)
Blank	1590214	12729	0.00800455	0.10		
0.01 std	1528370	2298.1	0.00150362	-0.07		
0.05 std	1288677	9511.6	0.00738093	0.09		
0.1 std	1389270	7609.2	0.00547711	0.04		
0.5 std	1476087	34494	0.02336832	0.51		
1 std	1497592	276076	0.1843468	4.80		
5 std	1404536	269008	0.19152816	5.00		
10 std	1475257	533471	0.36161252	9.53		
50 std	1486068	4E+06	2.41940678	64.39		
100 std	1502360	7E+06	4.94637903	131.76		
500 std	1516750	4E+07	24.2571617	646.61		
Blank	1336785	11957	0.00894464	0.13		
Blank	1448230	14098	0.00973477	0.15		
Blank	1455727	13276	0.00911999	0.13		
BLANK	1361690	13365	0.00981512	0.15		
1 check	1389671	267670	0.19261408	5.02		
BLANK	1475261	13146	0.00891098	0.13		
0DFB,3Bi,1	1500954	43445	0.02894503	0.66	66.08	0.28
0DFB,3Bi,2	1562229	48526	0.03106225	0.72	71.73	0.30
0DFB,3Bi,3	1414459	64462	0.04557326	1.10	110.41	0.46
0.1DFB,3Bi,1	1464024	95119	0.06497063	1.62	162.13	0.68
0.1DFB,3Bi,2	1404962	98749	0.07028594	1.76	176.30	0.74
0.1DFB,3Bi,3	1442435	103226	0.07156399	1.80	179.71	0.75
0.1DFB,3Bi,3	1515842	100826	0.06651505	1.66	166.25	0.70
0.2DFB,3Bi,1	1460841	123125	0.08428351	2.14	213.62	0.90
0.2DFB,3Bi,2	1821487	138136	0.0758371	1.91	191.10	0.80
0.2DFB,3Bi,3	1421758	137796	0.0969191	2.47	247.31	1.04
0DFB,10Bi,1	1411293	109651	0.07769528	1.96	196.05	0.82
0DFB,10Bi,2	1084649	122498	0.11293829	2.90	290.01	1.22
0DFB,10Bi,3	1549040	145993	0.09424728	2.40	240.18	1.01
0.1DFB,10Bi,1	1441362	123958	0.08600039	2.18	218.20	0.92
0.1DFB,10Bi,2	1447923	125169	0.08644735	2.19	219.39	0.92
0.1DFB,10Bi,3	1474839	135593	0.09193722	2.34	234.02	0.98
0.2DFB,10Bi 1	1455354	155380	0.10676433	2.74	273.55	1.15
0.2DFB,10Bi 2	1502831	167845	0.11168608	2.87	286.68	1.20
0.2DFB,10Bi 3	1441416	156419	0.10851746	2.78	278.23	1.17

Table C3. Raw ICP-MS data for total aqueous uranium from UO_2 dissolution with DFB (0mM, 0.1mM, or 0.2mM) with 3mM or 10mM bicarbonate after 21 days.

Sample ID	Bismuth / 209 [#3]	Uranium / 238 [#3]	U/Bi #3	U Conc. (ppb)	U Conc. (ppb)	U Conc. (μM)
0	239429.6	3696.22	0.0154376	0.1415763		
0.01	246019.2	776.72	0.0031572	-0.0739825		
0.05	246721.3	2718.17	0.0110172	0.0639843		
0.1	235155.5	2166.94	0.0092149	0.0323496		
0.5	231487.8	8912.29	0.0385	0.5463912		
1 Fe	239088.6	71272.18	0.2980994	5.1031396		
1	242156.7	14896.91	0.0615176	0.9504192		
5	232632.3	67972.78	0.2921898	4.9994072		
10	231416.8	138731	0.5994854	10.393368		
25/50	236588.1	674767.1	2.8520754	49.93308		
40/100	238487.4	2108542	8.841314	155.06219		
50/500	238630.5	10049010	42.111172	739.048		
Blank	239061.5	4418.72	0.0184836	0.1950428		
Blank	242754.9	4435.38	0.018271	0.1913112		
Blank	237089.6	4198.62	0.017709	0.1814461		
0DFB,3Bi,1	236219	10589.19	0.0448279	0.6574633	65.746326	0.2762103
0DFB,3Bi,2	237847	20839.63	0.0876178	1.4085552	140.85552	0.5917553
0DFB,3Bi,3	232417.4	11705.72	0.0503651	0.7546582	75.465818	0.3170433
0.1DFB,3Bi,1	239619.5	34482.43	0.1439049	2.3965634	239.65634	1.0068325
0.1DFB,3Bi,2	233989.2	37864	0.1618194	2.7110165	271.10165	1.138939
0.1DFB,3Bi,3	234705	36457.88	0.1553349	2.5971937	259.71937	1.0911203
0.1DFB,3Bi,3	232556.8	49148.75	0.2113408	3.5802658	358.02658	1.5041238
0.2DFB,3Bi,1	235402.5	61326.28	0.2605167	4.4434495	444.34495	1.8667603
0.2DFB,3Bi,2	241421.5	59843.8	0.247881	4.221655	422.1655	1.7735811
0.2DFB,3Bi,3	234763.1	57912.88	0.2466865	4.2006876	420.06876	1.7647723
0DFB,10Bi,1	250830.9	23459.74	0.0935281	1.5122989	151.22989	0.6353396
0DFB,10Bi,2	233249.6	37215.56	0.1595525	2.6712253	267.12253	1.1222221
0DFB,10Bi,3	236311.1	42904.21	0.1815582	3.0574905	305.74905	1.284498
0.1DFB,10Bi,1	236675.5	47625.26	0.201226	3.4027197	340.27197	1.429534
0.1DFB,10Bi,2	237303.1	53117.71	0.2238391	3.7996475	379.96475	1.5962894
0.1DFB,10Bi,3	237233.1	54226.84	0.2285804	3.882872	388.2872	1.6312532
0.2DFB,10Bi 1	239487.8	71204.36	0.2973194	5.0894468	508.94468	2.1381535
0.2DFB,10Bi 2	243117.3	77495.7	0.3187585	5.4657675	546.57675	2.2962515
0.2DFB,10Bi 3	240551.9	72851.85	0.3028529	5.1865776	518.65776	2.1789596
1 U	238485.4	15216.2	0.0638035	0.9905426		
Blank	250319.5	4228.63	0.0168929	0.1671216		

Table C4. Raw ICP-MS data for total aqueous uranium from UO_2 dissolution with DFB (0mM, 0.1mM, or 0.2mM) with 3mM or 10mM bicarbonate after 35 days.

Misc Info:	Bismuth / 209 [#2]	Uranium / 238 [#2]	U/Bi #2	U Conc. (ppb) #2	U Conc. in samples(ppb)	U Conc. in samples(μM)
0	1550310	12515.39	0.008073	0.083612		
0.01	1533052	3062.71	0.001998	-0.05118		
0.05	1542035	12262.9	0.007952	0.08094		
0.1	1484059	9553.86	0.006438	0.047332		
0.5	1559612	45151.78	0.028951	0.546828		
1 Fe	1560924	350725.2	0.224691	4.889714		
1	1536945	72449.6	0.047139	0.950366		
5	1526032	350624.8	0.229762	5.002239		
10	1571487	706945.8	0.449858	9.885497		
25/50	1520267	4632885	3.047415	67.5175		
40/100	1467590	9426387	6.423038	142.4125		
50/500	1426884	45387100	31.80854	705.6406		
Blank	1379548	17544.66	0.012718	0.186667		
Blank	1391027	17219.8	0.012379	0.179157		
Blank	1407747	11441.03	0.008127	0.084818		
0DFB,3Bi,1	1403617	54305.93	0.03869	0.762915	76.29148	0.320512
0DFB,3Bi,2	1443424	57747.29	0.040007	0.792139	79.21388	0.332789
0DFB,3Bi,3	1412369	50619.75	0.03584	0.699689	69.96891	0.29395
0.1DFB,3Bi,1	1430244	203342	0.142173	3.058891	305.8891	1.285086
0.1DFB,3Bi,2	1431630	211466.3	0.14771	3.181745	318.1745	1.336699
0.1DFB,3Bi,3	1426931	218158.4	0.152886	3.296591	329.6591	1.384948
0.1DFB,3Bi,3	1440407	331019.2	0.229809	5.003283	500.3283	2.101955
0.2DFB,3Bi,1	1432599	327461.3	0.228578	4.975971	497.5971	2.09048
0.2DFB,3Bi,2	1517428	415679.2	0.273937	5.982333	598.2333	2.513269
0.2DFB,3Bi,3	1453658	396344.6	0.272653	5.953858	595.3858	2.501306
0DFB,10Bi,1	1452949	125554.7	0.086414	1.821761	182.1761	0.765349
0DFB,10Bi,2	1441563	179766.1	0.124702	2.671268	267.1268	1.12224
0DFB,10Bi,3	1440807	199934.6	0.138766	2.983295	298.3295	1.253327
0.1DFB,10Bi,1	1443966	285833.9	0.197951	4.296429	429.6429	1.804995
0.1DFB,10Bi,2	1440151	333033.1	0.231249	5.035216	503.5216	2.11537
0.1DFB,10Bi,3	1429818	356819.9	0.249556	5.441403	544.1403	2.286015
0.2DFB,10Bi 1	1428547	499005.7	0.34931	7.65464	765.464	3.21583
0.2DFB,10Bi 2	1441170	507479.6	0.35213	7.717215	771.7215	3.242118
0.2DFB,10Bi 3	1443481	526754.8	0.36492	8.000976	800.0976	3.361331
1 U	1471053	69502.44	0.047247	0.952763		

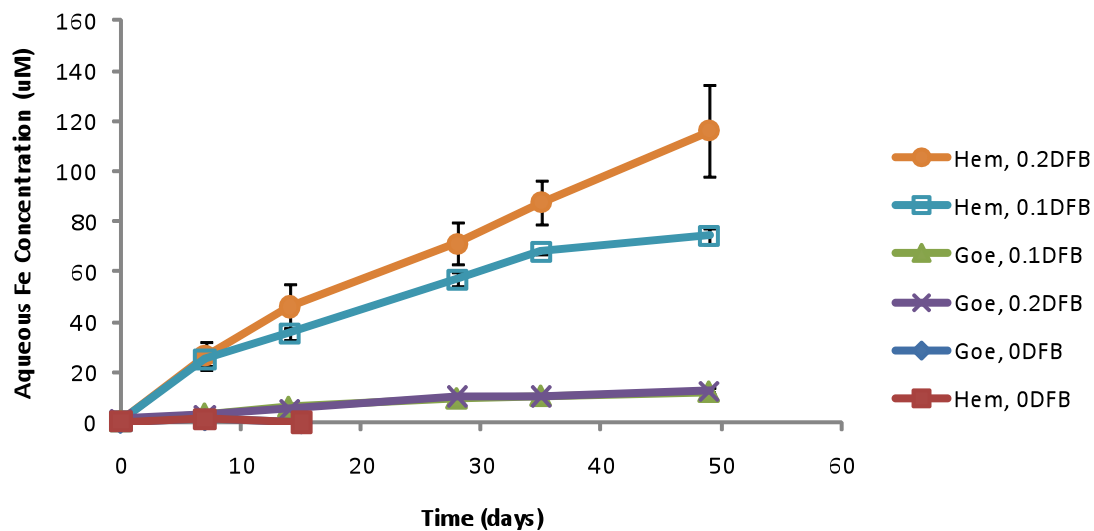


Figure C2. Dissolution of ferrihydrite, goethite, and hematite in the presence of DFB (0mM, 3mM, 10mM) with 0mM bicarbonate showing DFB promotes dissolution. This experiment was repeated on a longer time scale and shown in Chapter 3.

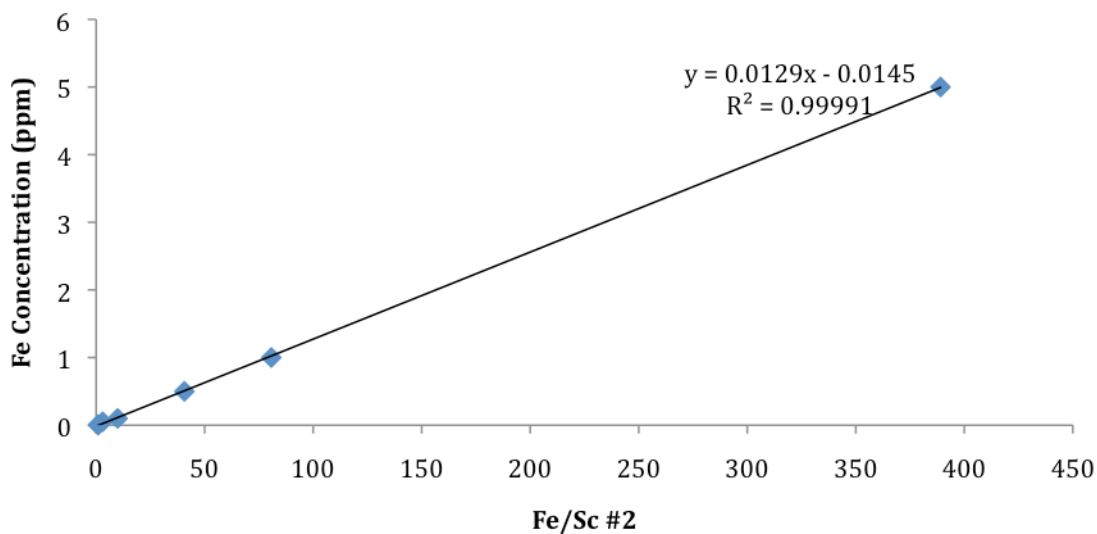


Figure C3. Iron calibration curve from ICP-MS data used for dissolution of iron minerals experiments. All calibration curves for iron were similar to this curve. A new calibration curve was made every time samples were run on the ICP-MS.

Table C5. Raw ICP-MS data for dissolution of ferrihydrite (F), goethite (G), and hematite (H) in the presence of DFB (0mM, 0.1mM, or 0.2mM) with 0mM bicarbonate.

Date	Days	Iron Oxide	DFB Conc. (mM)		Scandium / 45	Iron /56	Fe/Sc	Fe Conc. (ppm)	Fe Conc. in samp (ppm)	Fe Conc. in samp (μM)
4/6/10	0	F	0	1	116089.9	65332.09	0.563	0.005	0.016435	0.294272
				2	113298.4	38838.53	0.343	0.003	0.008252	0.147755
				3	112266.3	34539.1	0.308	0.002	0.006945	0.124346
			0.1	1	111662.1	44705.56	0.4	0.003	0.010394	0.186098
				2	112219.4	40751.63	0.363	0.003	0.009009	0.161305
				3	109035.4	39183.98	0.359	0.003	0.008869	0.158792
			0.2	1	109403.7	60772.24	0.555	0.005	0.016164	0.28942
				2	106742.1	43284.47	0.406	0.004	0.010585	0.189522
				3	108793.7	40394.03	0.371	0.003	0.009312	0.166732
		G	0	1	108107.7	78222.17	0.724	0.007	0.022416	0.401367
				2	109514.5	42402.98	0.387	0.003	0.009903	0.177323
				3	109824.6	37288.25	0.34	0.003	0.00813	0.145575
			0.1	1	111531.1	62001.19	0.556	0.005	0.01618	0.289702
				2	112816.9	59416.9	0.527	0.005	0.015092	0.270224
				3	112408.8	66500.37	0.592	0.006	0.017507	0.31347
			0.2	1	112369.2	51829.38	0.461	0.004	0.012658	0.226646
				2	113946.9	42732.68	0.375	0.003	0.009451	0.169218
				3	113894.3	44541.41	0.391	0.003	0.010048	0.179911
		H	0	1	113946.8	80134.6	0.703	0.007	0.021661	0.387849
				2	114176.4	33987.59	0.298	0.002	0.006574	0.1177
				3	114484.9	32284.65	0.282	0.002	0.00599	0.107258
			0.1	1	115005.1	42010.81	0.365	0.003	0.009089	0.162739
				2	111764.6	33986.32	0.304	0.002	0.006812	0.121971
				3	120820.6	33904.25	0.281	0.002	0.005939	0.106337
			0.2	1	116673.8	38068.98	0.326	0.003	0.007638	0.136756
				2	115785.7	35450.45	0.306	0.002	0.00689	0.12336
				3	117712.2	34129.11	0.29	0.002	0.006286	0.112545
4/26/10	20	F	0	1	120229.7	113201.6	0.942	0.01	0.030525	0.546561
				2	122223.4	48858.23	0.4	0.003	0.010371	0.185685
				3	123264.6	38123.85	0.309	0.002	0.007005	0.125432
			0.1	1	135565.6	13486530	99.48	1.232	3.696283	66.18233
				2	128441.1	13937870	108.5	1.344	4.032282	72.19843
				3	799711.9	2358290	2.949	0.035	0.1052	1.883617
			0.2	1	130314.8	27254590	209.1	2.592	7.775666	139.2241
				2	131078.2	28101050	214.4	2.657	7.970579	142.714
				3	125952.9	28775340	228.5	2.831	8.494253	152.0905
		G	0	1	121743.4	124495	1.023	0.011	0.033541	0.600551
				2	119806.9	43614.98	0.364	0.003	0.009042	0.161906
				3	121010.9	38536.08	0.318	0.002	0.007346	0.131538
			0.1	1	131173.7	1735306	13.23	0.163	0.487621	8.730911
				2	128560.6	1715641	13.34	0.164	0.491934	8.808128
				3	129857.2	1708530	13.16	0.162	0.48494	8.682902
			0.2	1	130278.9	1909644	14.66	0.18	0.540782	9.68276
				2	131822.5	1960627	14.87	0.183	0.548784	9.82604
				3	133062	1898311	14.27	0.175	0.526209	9.421822
		H	0	1	122660.4	128224.8	1.045	0.011	0.034388	0.615713
				2	122037.6	59232.5	0.485	0.005	0.013555	0.242712
				3	116531.5	49228.26	0.422	0.004	0.011215	0.200806
			0.1	1	133037.6	6764457	50.85	0.629	1.886979	33.78655
				2	132768.7	6229475	46.92	0.58	1.740915	31.17126
				3	136483.5	6215743	45.54	0.563	1.689666	30.25364
			0.2	1	133777.1	6068670	45.36	0.561	1.683042	30.13505

Table C5. Continued

Date	Days	Iron Oxide	DFB Conc. (mM)	Scandium / 45	Iron /56	Fe/Sc	Fe Conc. (ppm)	Fe Conc. in samp (ppm)	Fe Conc. in samp (µM)		
5/16/10	40	F	0	2	131799.1	6368397	48.32	0.598	1.792966	32.10324	
				3	133263.2	6548546	49.14	0.608	1.823506	32.65006	
				1	115590.5	28846.44	0.25	0.01	0.029984	0.536868	
				2	117579.8	36755.83	0.313	0.011	0.032254	0.577506	
				3	114805.4	30822.04	0.268	0.01	0.030665	0.54906	
				0.1	1	112437	11083380	98.57	1.19	3.569669	63.91529
			2	112241	11723840	104.5	1.26	3.781286	67.70431		
			3	112644.2	11460920	101.7	1.228	3.6838	65.95881		
			0.2	1	111916	23143250	206.8	2.488	7.465485	133.6703	
			2	111304.3	23066490	207.2	2.494	7.481571	133.9583		
			3	109783.7	23141580	210.8	2.537	7.60953	136.2494		
		G	0	1	110075.4	28797.54	0.262	0.01	0.030418	0.544641	
				2	111218.5	32606.67	0.293	0.011	0.031554	0.564984	
				3	113228.4	37273.41	0.329	0.011	0.032851	0.588196	
			0.1	1	115386	1983745	17.19	0.213	0.639921	11.45785	
				2	113936.3	2015693	17.69	0.219	0.657891	11.7796	
				3	113413.1	2017870	17.79	0.221	0.66152	11.84458	
			0.2	1	113044.1	2216941	19.61	0.242	0.727007	13.01713	
				2	113636.7	2250137	19.8	0.245	0.733841	13.1395	
				3	113794.1	2214613	19.46	0.241	0.721617	12.92062	
			H	0	1	113731.2	27550.67	0.242	0.01	0.029721	0.532154
					2	114783.9	33600.01	0.293	0.011	0.031538	0.564692
					3	115225.1	30282.61	0.263	0.01	0.030461	0.545412
				0.1	1	112394.3	8025648	71.41	0.864	2.591623	46.40327
					2	114388.4	7608507	66.51	0.805	2.415528	43.25028
					3	112017.2	7549716	67.4	0.816	2.447322	43.81955
6/5/10	60	F	0	1	113465.5	7606175	67.04	0.811	2.434265	43.58576	
				2	112667.8	8015708	71.14	0.861	2.582206	46.23467	
				3	112356.4	8034298	71.51	0.865	2.595261	46.46842	
			0.1	1	98240.59	32303.72	0.329	0.015	0.044952	0.804879	
				2	100466.9	37378.29	0.372	0.015	0.046366	0.830186	
				3	99697.97	33030.81	0.331	0.015	0.045034	0.806335	
			0.2	1	105322.2	11007470	104.5	1.151	3.451754	61.804	
				2	101739.8	11552290	113.5	1.249	3.7472	67.094	
				3	104964.8	11378720	108.4	1.193	3.579047	64.0832	
			G	0	1	102843	22815640	221.8	2.43	7.28867	130.5044
					2	103751.7	23160550	223.2	2.445	7.333839	131.3131
					3	102593.6	23396170	228	2.497	7.491339	134.1332
				H	0	1	95386.34	30234.72	0.317	0.015	0.044565
			2			95483.2	30653.38	0.321	0.015	0.044698	0.800319
		3	94720.6			32047.49	0.338	0.015	0.045264	0.81045	
		0.1	1		103683.4	2317358	22.35	0.255	0.765056	13.6984	
			2		104490.5	2357015	22.56	0.257	0.771821	13.81954	
			3		102883.6	2379990	23.13	0.264	0.790644	14.15656	
			0.2		1	104178	2692983	25.85	0.293	0.879489	15.74735
		G	0	2	101906.9	2586961	25.39	0.288	0.864307	15.4755	
				3	103628.8	2560880	24.71	0.281	0.842284	15.08118	
				1	98172.42	27419.12	0.279	0.014	0.043333	0.775881	
				2	98223.8	29509.88	0.3	0.015	0.044024	0.788258	
				3	97153.5	31371.61	0.323	0.015	0.044759	0.801416	
				0.1	1	102637.5	9350699	91.1	1.004	3.013305	53.95353
				2	102568.2	9096273	88.69	0.978	2.934203	52.53721	
			0.2	3	103007.6	9555235	92.76	1.023	3.067531	54.92447	
				1	103533.8	9410700	90.89	1.002	3.006465	53.83107	

Table C5. Continued

		Iron	DFB			Fe	Fe Conc.	Fe Conc.		
Date	Days	Oxide	Conc.	Scandium	Iron /56	Fe/Sc	Conc.	in samp	in samp	
			(mM)	/ 45			(ppm)	(ppm)	(μM)	
6/25/10	80	F	0	2	133896.5	10311910	77.01	0.851	2.552559	45.70383
				3	105257.7	10109800	96.05	1.058	3.174972	56.8482
				1	1473472	105407.2	0.072	0.076	0.227555	4.074393
				2	1429167	104817.2	0.073	0.079	0.238375	4.268124
				3	1450682	107626.4	0.074	0.081	0.243463	4.359228
				0.1	1	1404711	232500.7	0.166	0.264	0.790946
			2	1429725	244797.4	0.171	0.275	0.825146	14.77433	
			3	1331676	238844.8	0.179	0.291	0.873925	15.64771	
			0.2	1	1427197	2174653	1.524	2.978	8.933267	159.9511
			2	1408811	2151877	1.527	2.985	8.955562	160.3503	
			3	1385409	2157843	1.558	3.045	9.136053	163.582	
		G	0	1	1384794	164682.5	0.119	0.171	0.511626	9.160709
				2	1406466	97555.02	0.069	0.072	0.214517	3.84095
				3	1428403	96120.34	0.067	0.067	0.20211	3.618798
			0.1	1	1439524	235820	0.164	0.26	0.780773	13.97982
				2	1429765	244257.2	0.171	0.274	0.822853	14.73326
				3	1410240	233319.5	0.165	0.264	0.790536	14.15463
			0.2	1	1416801	256243.7	0.181	0.294	0.882942	15.80917
				2	1433405	253231	0.177	0.286	0.857783	15.35869
				3	1445329	248787.4	0.172	0.277	0.830614	14.87223
		H	0	1	1420311	95728.52	0.067	0.068	0.202754	3.630338
				2	1416745	96470.87	0.068	0.069	0.206913	3.704792
				3	1407275	94637.03	0.067	0.067	0.201848	3.614102
			0.1	1	1450564	1072176	0.739	1.41	4.229796	75.73493
				2	1440322	1069681	0.743	1.417	4.25092	76.11316
				3	1470359	1123662	0.764	1.46	4.380058	78.42539
			0.2	1	1443326	1187774	0.823	1.577	4.732157	84.72975
				2	1437451	1230757	0.856	1.644	4.931581	88.30047
				3	1447898	1210476	0.836	1.604	4.810574	86.13383

Table C6. Raw ICP-MS data for dissolution of ferrihydrite (F), goethite (G), and hematite (H) in the presence of citrate (0mM, 0.1mM, or 0.2mM) with 0mM bicarbonate.

Date	Days	Iron Oxide	Citr. Conc. (mM)		Scandium /45	Iron/56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (μM)
4/6/10	0	F	0	1	116090	65332	0.5628	0.005	0.0164	0.2943
				2	113298	38839	0.3428	0.003	0.0083	0.1478
				3	112266	34539	0.3077	0.002	0.0069	0.1243
			0.1	1	115930	83233	0.718	0.007	0.0222	0.3976
				2	117634	59900	0.5092	0.005	0.0144	0.2586
				3	117677	81741	0.6946	0.007	0.0213	0.3821
			0.2	1	118718	72455	0.6103	0.006	0.0182	0.3259
				2	117966	56194	0.4764	0.004	0.0132	0.2367
				3	119831	80794	0.6742	0.007	0.0206	0.3685
		G	0	1	108108	78222	0.7236	0.007	0.0224	0.4014
				2	109515	42403	0.3872	0.003	0.0099	0.1773
				3	109825	37288	0.3395	0.003	0.0081	0.1456
			0.1	1	119110	107751	0.9046	0.010	0.0292	0.522
				2	109671	63151	0.5758	0.006	0.0169	0.303
				3	119383	61512	0.5153	0.005	0.0147	0.2626
			0.2	1	119267	70766	0.5933	0.006	0.0176	0.3146
				2	122422	88855	0.7258	0.008	0.0225	0.4029
				3	120715	40363	0.3344	0.003	0.0079	0.1421
		H	0	1	113947	80135	0.7033	0.007	0.0217	0.3878
				2	114176	33988	0.2977	0.002	0.0066	0.1177
				3	114485	32285	0.282	0.002	0.006	0.1073
			0.1	1	121657	51349	0.4221	0.004	0.0112	0.2006
				2	120317	35378	0.294	0.002	0.0064	0.1153
				3	121022	36749	0.3037	0.002	0.0068	0.1217
			0.2	1	122580	40281	0.3286	0.003	0.0077	0.1383
				2	120905	35872	0.2967	0.002	0.0065	0.117
				3	122083	35831	0.2935	0.002	0.0064	0.1149
4/26/10	20	F	0	1	120230	113202	0.9415	0.010	0.0305	0.5466
				2	122223	48858	0.3997	0.003	0.0104	0.1857
				3	123265	38124	0.3093	0.002	0.007	0.1254
			0.1	1	127590	282657	2.2153	0.026	0.0779	1.395
				2	127955	226649	1.7713	0.020	0.0614	1.0992
				3	122373	241137	1.9705	0.023	0.0688	1.2319
			0.2	1	131559	2E+06	12.373	0.152	0.4558	8.1606
				2	130005	2E+06	15.184	0.187	0.5603	10.033
				3	130114	2E+06	14.372	0.177	0.5302	9.4924
		G	0	1	121743	124495	1.0226	0.011	0.0335	0.6006
				2	119807	43615	0.364	0.003	0.009	0.1619
				3	121011	38536	0.3185	0.002	0.0073	0.1315
			0.1	1	123275	224535	1.8214	0.021	0.0633	1.1326
				2	123968	143800	1.16	0.013	0.0387	0.6921
				3	121188	144088	1.189	0.013	0.0397	0.7114
			0.2	1	123992	189688	1.5298	0.017	0.0524	0.9384
				2	124421	229226	1.8424	0.021	0.064	1.1466
				3	125888	359851	2.8585	0.034	0.1018	1.8234
		H	0	1	122660	128225	1.0454	0.011	0.0344	0.6157
				2	122038	59233	0.4854	0.005	0.0136	0.2427
				3	116532	49228	0.4224	0.004	0.0112	0.2008
			0.1	1	126329	1E+06	9.5875	0.117	0.3522	6.3054
				2	137810	1E+06	9.3196	0.114	0.3422	6.127
				3	123331	936207	7.591	0.093	0.2779	4.9756
			0.2	1	128555	930854	7.2409	0.088	0.2649	4.7424
				2	126834	1E+06	10.484	0.129	0.3855	6.9027

Table C6. Continued

Date	Days	Iron Oxide	Citr. Conc (mM)		Scandium /45	Iron/56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (μ M)
5/16/10	40	F	0	3	130937	2E+06	12.22	0.150	0.4501	8.0588
				1	115591	28846	0.2496	0.010	0.03	0.5369
				2	117580	36756	0.3126	0.011	0.0323	0.5775
			0.1	3	114805	30822	0.2685	0.010	0.0307	0.5491
				1	116266	198752	1.7095	0.028	0.0825	1.4779
				2	117037	202998	1.7345	0.028	0.0834	1.494
				3	114742	249039	2.1704	0.033	0.0991	1.775
			0.2	1	114229	2E+06	18.507	0.229	0.6873	12.306
				2	120697	2E+06	19.185	0.237	0.7117	12.742
				3	126585	2E+06	17.295	0.215	0.6436	11.524
		G	0	1	110075	28798	0.2616	0.010	0.0304	0.5446
				2	111219	32607	0.2932	0.011	0.0316	0.565
				3	113228	37273	0.3292	0.011	0.0329	0.5882
			0.1	1	115874	239265	2.0649	0.032	0.0953	1.707
				2	116095	257224	2.2156	0.034	0.1008	1.8042
				3	113727	230915	2.0304	0.031	0.0941	1.6848
			0.2	1	113727	261248	2.2971	0.035	0.1037	1.8567
				2	115204	685035	5.9463	0.078	0.2351	4.2089
				3	115330	2E+06	14.547	0.182	0.5447	9.7528
		H	0	1	113731	27551	0.2422	0.010	0.0297	0.5322
				2	114784	33600	0.2927	0.011	0.0315	0.5647
				3	115225	30283	0.2628	0.010	0.0305	0.5454
			0.1	1	114715	2E+06	21.28	0.262	0.7871	14.093
				2	116090	690109	5.9446	0.078	0.235	4.2078
				3	115779	487437	4.2101	0.058	0.1726	3.0898
			0.2	1	111334	2E+06	16.355	0.203	0.6098	10.918
				2	116139	2E+06	19.641	0.243	0.7281	13.036
				3	115492	2E+06	21.592	0.266	0.7983	14.294
6/5/10	60	F	0	1	98241	32304	0.3288	0.015	0.045	0.8049
				2	100467	37378	0.372	0.015	0.0464	0.8302
				3	99698	33031	0.3313	0.015	0.045	0.8063
			0.1	1	101751	180127	1.7703	0.031	0.0921	1.6488
				2	102061	212640	2.0835	0.034	0.1023	1.8322
				3	103464	243409	2.3526	0.037	0.1111	1.9898
			0.2	1	102328	2E+06	22.929	0.261	0.784	14.037
				2	104188	3E+06	24.656	0.280	0.8405	15.048
				3	103386	2E+06	24.044	0.273	0.8204	14.69
		G	0	1	95386	30235	0.317	0.015	0.0446	0.7979
				2	95483	30653	0.321	0.015	0.0447	0.8003
				3	94721	32047	0.3383	0.015	0.0453	0.8104
			0.1	1	99561	361601	3.632	0.051	0.153	2.7389
				2	99051	801900	8.0958	0.100	0.2989	5.3524
				3	99529	664801	6.6795	0.084	0.2526	4.5232
			0.2	1	100443	345431	3.4391	0.049	0.1467	2.6259
				2	103022	1E+06	11.528	0.137	0.4112	7.3617
				3	101314	2E+06	24.189	0.275	0.8252	14.775
		H	0	1	98172	27419	0.2793	0.014	0.0433	0.7759
				2	98224	29510	0.3004	0.015	0.044	0.7883
				3	97154	31372	0.3229	0.015	0.0448	0.8014
			0.1	1	102288	3E+06	29.367	0.331	0.9945	17.806
				2	102960	328949	3.1949	0.046	0.1387	2.483
				3	104009	172460	1.6581	0.029	0.0884	1.5832
			0.2	1	103442	3E+06	25.111	0.285	0.8553	15.315
				2	101750	3E+06	30.999	0.349	1.0479	18.762
				3	102586	3E+06	29.665	0.335	1.0042	17.981
6/25/10	80	F	0	1	1E+06	105407	0.0715	0.076	0.2276	4.0744

Table C6. Continued

								Fe	Fe
		Iron	Citr.	Scandium			Fe	Conc in	Conc in
Date	Days	Oxide	Conc (mM)	/45	Iron/56	Fe/Sc	Conc. (ppm)	samp (ppm)	samp (μM)
			2	1E+06	104817	0.0733	0.079	0.2384	4.2681
			3	1E+06	107626	0.0742	0.081	0.2435	4.3592
			0.1	1	1E+06	105407	0.0715	0.076	4.0744
			2	1E+06	104817	0.0733	0.079	0.2384	4.2681
			3	1E+06	107626	0.0742	0.081	0.2435	4.3592
			0.2	1	1E+06	232501	0.1655	0.264	14.162
			2	1E+06	244797	0.1712	0.275	0.8251	14.774
			3	1E+06	238845	0.1794	0.291	0.8739	15.648
		G	0	1	1E+06	164683	0.1189	0.171	9.1607
			2	1E+06	97555	0.0694	0.072	0.2145	3.841
			3	1E+06	96120	0.0673	0.067	0.2021	3.6188
			0.1	1	1E+06	141447	0.0962	0.125	6.722
			2	1E+06	169054	0.1133	0.159	0.4778	8.5547
			3	1E+06	136167	0.0941	0.121	0.363	6.4993
			0.2	1	2E+06	120227	0.0801	0.093	4.9969
			2	1E+06	150252	0.1033	0.139	0.4182	7.4879
			3	1E+06	270190	0.1824	0.297	0.8921	15.972
		H	0	1	1E+06	95729	0.0674	0.068	3.6303
			2	1E+06	96471	0.0681	0.069	0.2069	3.7048
			3	1E+06	94637	0.0672	0.067	0.2018	3.6141
			0.1	1	1E+06	239671	0.1604	0.253	13.616
			2	1E+06	115787	0.0782	0.089	0.2674	4.7883
			3	1E+06	102637	0.0698	0.072	0.2171	3.8863
			0.2	1	1E+06	188494	0.1275	0.188	10.081
			2	1E+06	303090	0.2075	0.347	1.0424	18.665
			3	1E+06	271426	0.1885	0.309	0.9284	16.624

Table C7. Raw ICP-MS data for dissolution of ferrihydrite (F), goethite (G), and hematite (H) in the presence of EDTA (0mM, 0.1mM, or 0.2mM) with 10mM bicarbonate.

Date	Days	Iron Oxide	EDTA Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
6/2/10	0	F	0	1	85615.63	54664.11	0.64	0	0	0
				2	85583.73	56463.76	0.66	0	0	0
				3	85714.05	46208.76	0.54	0	0	0
			0.1	1	85672.68	72036.98	0.84	0	0	0
				2	85544.8	77170.68	0.90	0	0	0
				3	84498.68	62544.24	0.74	0	0	0
			0.2	1	85722.41	76389.16	0.89	0	0	0
				2	86848.32	103064.5	1.19	0.001	0.002	0.043
				3	86591.3	63472.76	0.73	0	0	0
		G	0	1	86250.04	62709.47	0.73	0	0	0
				2	89016.38	44700.29	0.50	0	0	0
				3	89236.7	33150.66	0.37	0	0	0
			0.1	1	89122.82	35665.92	0.40	0	0	0
				2	89983.54	35248.56	0.39	0	0	0
				3	89782.49	48020.88	0.53	0	0	0
			0.2	1	89941.26	55540.93	0.62	0	0	0
				2	89756.5	46573.19	0.52	0	0	0
				3	88036.17	38134.99	0.43	0	0	0
		H	0	1	89297.34	56190.7	0.63	0	0	0
				2	89960.28	34863.02	0.39	0	0	0
				3	89693.99	38570.8	0.43	0	0	0
			0.1	1	90826.27	46850.3	0.52	0	0	0
				2	91467.53	35343.03	0.39	0	0	0
				3	90664.17	64748.85	0.71	0	0	0
			0.2	1	90210.4	37970.96	0.42	0	0	0
				2	91561.2	37302.42	0.41	0	0	0
				3	91993.52	46067.37	0.50	0	0	0
6/28/10	20	F	0	1	92353.7	154074.6	1.67	0.007	0.021	0.377
				2	93017.04	67721.96	0.73	0	0	0
				3	93736.08	66228.34	0.71	0	0	0
			0.1	1	92305.74	11008410	119.26	1.524	4.572	81.860
				2	92777.44	11514030	124.10	1.586	4.759	85.216
				3	92218.39	11128110	120.67	1.542	4.626	82.838
			0.2	1	91422.55	21885860	239.39	3.074	9.221	165.103
				2	91888.5	21982930	239.23	3.072	9.215	164.994
				3	92541.41	22236470	240.29	3.085	9.256	165.722
		G	0	1	93935.87	229894.3	2.45	0.017	0.051	0.917
				2	92122.32	215545.3	2.34	0.016	0.047	0.842
				3	93424.31	62696.19	0.67	0	0	0
			0.1	1	93684.29	2054368	21.93	0.268	0.805	14.416

Table C7. Continued

Table 67: Continued										
Date	Days	Iron Oxide	EDTA Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
7/18/10	40	H	0.2	2	93815.44	2510340	26.76	0.331	0.992	17.763
				3	91791.86	2222823	24.22	0.298	0.894	16.001
				1	92406.09	2257996	24.44	0.301	0.902	16.153
				2	80256.95	2255180	28.10	0.348	1.044	18.692
				3	94245.65	2804299	29.76	0.369	1.108	19.839
				1	92588.73	74709.47	0.81	0	0	0
			0	2	92233.63	51800.12	0.56	0	0	0
				3	92903.1	52420.06	0.56	0	0	0
				1	94792.63	5975914	63.04	0.799	2.396	42.905
			0.1	2	93545.69	6688130	71.50	0.908	2.723	48.763
				3	92596.53	6711926	72.49	0.921	2.762	49.448
				1	92225.05	8204295	88.96	1.133	3.399	60.864
			0.2	2	93702.23	7528154	80.34	1.022	3.066	54.892
				3	93042.96	7955307	85.50	1.088	3.265	58.467
				1	93946.88	143697.5	1.53	0.022	0.067	1.199
		F	0	2	94570.39	64205.8	0.68	0.012	0.035	0.628
				3	92893.8	74838.71	0.81	0.013	0.040	0.713
				1	103522.3	11659590	112.63	1.489	4.467	79.985
			0.1	2	103882.6	11827470	113.85	1.505	4.516	80.861
				3	103818.8	11842900	114.07	1.508	4.525	81.017
				1	103722.6	23549830	227.05	3.011	9.032	161.727
			0.2	2	102976.4	23467920	227.90	3.022	9.066	162.334
				3	101473.2	23251110	229.14	3.039	9.116	163.219
				1	91622.41	52540.49	0.57	0.010	0.031	0.557
			0	2	90112.96	54663.17	0.61	0.011	0.032	0.579
				3	94068.99	55505.89	0.59	0.011	0.032	0.568
				1	94361.92	3171961	33.61	0.423	1.270	22.742
			0.1	2	94084.66	3334597	35.44	0.446	1.339	23.969
				3	94274.72	3379213	35.84	0.451	1.354	24.239
				1	96056.22	3461518	36.04	0.454	1.361	24.368
		G	0.2	2	94293.91	3402687	36.09	0.454	1.363	24.402
				3	87964.81	4429065	50.35	0.633	1.898	33.979
				1	89107.98	68073.77	0.76	0.013	0.038	0.685
			0	2	89507.32	56841.33	0.64	0.011	0.033	0.598
				3	90047.47	55375.66	0.61	0.011	0.033	0.585
				1	96979.82	8631397	89.00	1.116	3.347	59.932
			0.1	2	96243.04	9924292	103.12	1.292	3.876	69.409
				3	96952.3	10104410	104.22	1.306	3.918	70.150
				1	97431.88	13444640	137.99	1.728	5.184	92.824
			0.2	2	96280.23	12769520	132.63	1.661	4.983	89.224
				3	97235.07	13140490	135.14	1.692	5.077	90.911
1	53952.77			64223.64	1.19	0.013	0.038	0.683		

Table C7. Continued

Date	Days	Iron Oxide	EDTA Conc (mM)	Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
8/30/10	83	G	0.1	2	53576.87	41511.55	0.77	0.008	0.024
				3	53844.54	40901.77	0.76	0.008	0.023
				1	49315.38	5679740	115.17	1.335	4.005
				2	48926.37	5797468	118.49	1.373	4.120
				3	48947.41	5791205	118.31	1.371	4.114
				1	48886.12	11457640	234.37	2.718	8.153
			0.2	2	49055.64	11471480	233.85	2.712	8.135
				3	49141.32	11800210	240.13	2.784	8.353
				1	54188.26	39080.15	0.72	0.007	0.022
			0	2	54213.86	35801.69	0.66	0.007	0.020
				3	53879.22	30144.32	0.56	0.005	0.016
				1	51503.99	2276339	44.20	0.512	1.535
			0.1	2	50723.29	2440408	48.11	0.557	1.671
				3	51131.27	2453492	47.98	0.556	1.667
				1	50532.87	2499655	49.47	0.573	1.718
			0.2	2	50669.98	2458298	48.52	0.562	1.685
				3	51340.13	3256999	63.44	0.735	2.204
				1	59198.48	40825.07	0.69	0.007	0.021
		H	0	2	55863.18	29844.87	0.53	0.005	0.015
				3	55264.49	29580.88	0.54	0.005	0.015
				1	50692.21	5234232	103.26	1.197	3.590
			0.1	2	50734.49	5847940	115.27	1.336	4.008
				3	49647.56	5857776	117.99	1.368	4.103
				1	49921.8	8555061	171.37	1.987	5.960
			0.2	2	49334.18	8827547	178.93	2.075	6.224
				3	49664.5	8574801	172.65	2.002	6.005
				1	60757.04	77351.55	1.27	0.011	0.033
			0	2	61572.1	42574.34	0.69	0.004	0.013
				3	60810.64	44723.2	0.74	0.005	0.014
				1	62185.51	6951460	111.79	1.326	3.979
			0.1	2	66507.24	7285532	109.54	1.300	3.899
				3	61503.7	7156954	116.37	1.381	4.142
				1	62423.93	14232440	228.00	2.709	8.127
			0.2	2	60801.92	14092880	231.78	2.754	8.263
				3	61031.76	14217320	232.95	2.768	8.304
				1	59135.15	36967.02	0.63	0.003	0.010
		G	0	2	62066.04	29113.37	0.47	0.002	0.005
				3	59659.38	31018.39	0.52	0.002	0.007
				1	62712.14	3342172	53.29	0.630	1.891
			0.1	2	62679.91	3615860	57.69	0.682	2.047
				3	65821.26	3566040	54.18	0.641	1.922
				1	62125.08	3739224	60.19	0.712	2.137

Table C7. Continued

Date	Days	Iron Oxide	EDTA Conc (mM)	Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)	
				2	61969.1	3649670	58.89	0.697	2.091	37.432
				3	61493.83	4778492	77.71	0.921	2.762	49.456
		H	0	1	59783.23	58442.63	0.98	0.008	0.023	0.410
				2	59557.77	46946.67	0.79	0.005	0.016	0.289
				3	58884.05	33369.22	0.57	0.003	0.008	0.147
			0.1	1	62267.91	6386246	102.56	1.216	3.649	65.343
				2	67351.55	7043354	104.58	1.240	3.721	66.631
				3	62184.34	7232820	116.31	1.380	4.140	74.134
			0.2	1	62203.23	12292130	197.61	2.348	7.043	126.101
				2	62617.21	12684460	202.57	2.407	7.220	129.271
				3	62535.98	12363420	197.70	2.349	7.046	126.158

Table C8. Raw ICP-MS data for dissolution of ferrihydrite (F), goethite (G), and hematite (H) in the presence of NTA (0mM, 0.1mM, or 0.2mM) with 10mM bicarbonate.

Date	Days	Iron Oxide	NTA. Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
6/2/10	0	F	0	1	85615.63	54664.11	0.638	0	0	0
				2	85583.73	56463.76	0.660	0	0	0
				3	85714.05	46208.76	0.539	0	0	0
			0.1	1	91674.07	60807.11	0.663	0	0	0
				2	91189.89	63065.33	0.692	0	0	0
				3	92639.02	48566.83	0.524	0	0	0
			0.2	1	90824.27	57003.14	0.628	0	0	0
				2	92282.23	48527.69	0.526	0	0	0
				3	92056.13	63111.28	0.686	0	0	0
		G	0	1	86250.04	62709.47	0.727	0	0	0
				2	89016.38	44700.29	0.502	0	0	0
				3	89236.7	33150.66	0.371	0	0	0
			0.1	1	92602.91	55086.68	0.595	0	0	0
				2	93073.88	46305.08	0.498	0	0	0
				3	92775.45	29409.18	0.317	0	0	0
			0.2	1	91618.34	28917.86	0.316	0	0	0
				2	92416.73	29344.95	0.318	0	0	0
				3	91051.3	48215.15	0.530	0	0	0
		H	0	1	89297.34	56190.7	0.629	0	0	0
				2	89960.28	34863.02	0.388	0	0	0
				3	89693.99	38570.8	0.430	0	0	0
			0.1	1	92801.94	43836.14	0.472	0	0	0
				2	93723.59	63569.82	0.678	0	0	0
				3	94269.38	31003.39	0.329	0	0	0
			0.2	1	92913.8	29650.45	0.319	0	0	0
				2	92675.83	27231.11	0.294	0	0	0
				3	93847.76	28554.57	0.304	0	0	0
6/28/10	20	F	0	1	92353.7	154074.6	1.668	0.007	0.021	0.377
				2	93017.04	67721.96	0.728	0	0	0
				3	93736.08	66228.34	0.707	0	0	0
			0.1	1	95147.35	1133280	11.911	0.139	0.417	7.474
				2	93423.93	1022229	10.942	0.127	0.380	6.803
				3	94053.66	1169127	12.430	0.146	0.438	7.835
			0.2	1	93759.55	2805142	29.918	0.371	1.114	19.952
				2	93383.48	2466748	26.415	0.326	0.979	17.525
				3	93842.28	2458796	26.201	0.323	0.970	17.377
		G	0	1	93935.87	229894.3	2.447	0.017	0.051	0.917
				2	92122.32	215545.3	2.340	0.016	0.047	0.842
				3	93424.31	62696.19	0.671	0	0	0
			0.1	1	93035.83	81438.8	0.875	0	0	0
				2	93779.23	59677.11	0.636	0	0	0

Table C8. Continued

Date	Days	Iron Oxide	NTA. Conc (mM)	Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
7/18/10	40	H	0.2	3	93367.08	84873.13	0.909	0	0
				1	93096.06	95390.55	1.025	0	0
				2	93978.47	165586.6	1.762	0.008	0.025
			0	3	93047.95	81756.96	0.879	0	0
				1	92588.73	74709.47	0.807	0	0
				2	92233.63	51800.12	0.562	0	0
			0.1	3	92903.1	52420.06	0.564	0	0
				1	96026.18	111438.5	1.161	0.000	0.001
				2	92331.61	93505.13	1.013	0	0
			0.2	3	93769.5	84994.19	0.906	0	0
				1	92807.7	118290.2	1.275	0.002	0.006
				2	94271.66	110317.2	1.170	0.001	0.002
		F	0	3	95469.07	127750.4	1.338	0.003	0.008
				1	93946.88	143697.5	1.530	0.022	0.067
				2	94570.39	64205.8	0.679	0.012	0.035
			0.1	3	92893.8	74838.71	0.806	0.013	0.040
				1	95315.95	1088219	11.417	0.146	0.438
				2	92407.23	1014781	10.982	0.140	0.421
			0.2	3	93600.34	1063625	11.363	0.145	0.436
				1	90848.18	2469849	27.187	0.343	1.029
				2	95573.17	2464906	25.791	0.326	0.977
		G	0	3	95077.16	2462737	25.903	0.327	0.981
				1	91622.41	52540.49	0.573	0.010	0.031
				2	90112.96	54663.17	0.607	0.011	0.032
			0.1	3	94068.99	55505.89	0.590	0.011	0.032
				1	93916.66	109167.5	1.162	0.018	0.053
				2	94428.07	73523.38	0.779	0.013	0.039
			0.2	3	94556.69	59531.51	0.630	0.011	0.033
				1	94793.17	97774.47	1.031	0.016	0.048
				2	96291.06	126617.8	1.315	0.020	0.059
		H	0	3	94279.27	138424.3	1.468	0.022	0.065
				1	89107.98	68073.77	0.764	0.013	0.038
				2	89507.32	56841.33	0.635	0.011	0.033
			0.1	3	90047.47	55375.66	0.615	0.011	0.033
				1	94606.65	108608	1.148	0.018	0.053
				2	93920.13	94316.77	1.004	0.016	0.047
			0.2	3	94023.26	111999.7	1.191	0.018	0.054
				1	95062	131125.9	1.379	0.020	0.061
				2	95802.3	133719.7	1.396	0.021	0.062
		8/7/10	60	3	95068.56	157522.4	1.657	0.024	0.072
				1	53952.77	64223.64	1.190367798	0.013	0.038
				2	53576.87	41511.55	0.774803567	0.008	0.024

Table C8. Continued

Date	Days	Iron Oxide	NTA. Conc (mM)	Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)		
8/30/10	83	G	0.1	3	53844.54	40901.77	0.759627067	0.008	0.023	0.414	
				1	53129.71	412450	7.763076441	0.089	0.267	4.778	
				2	53702.92	372326.7	6.933081106	0.079	0.238	4.261	
			0.2	3	53551.41	392886.7	7.336626617	0.084	0.252	4.512	
				1	53056.15	1376114	25.93693662	0.300	0.899	16.102	
				2	50863.88	1312784	25.80974947	0.298	0.895	16.023	
			0	3	52275.62	1316682	25.18730529	0.291	0.873	15.635	
				1	54188.26	39080.15	0.721192192	0.007	0.022	0.390	
				2	54213.86	35801.69	0.660378914	0.007	0.020	0.352	
			0.1	3	53879.22	30144.32	0.559479517	0.005	0.016	0.290	
				1	53554.63	38414.14	0.717288869	0.007	0.022	0.388	
				2	52729.27	39028.85	0.74017429	0.007	0.022	0.402	
			0.2	3	53409.7	39931.42	0.747643593	0.008	0.023	0.407	
				1	53533.5	42752.92	0.79861993	0.008	0.024	0.439	
				2	53144.5	64266.48	1.2092781	0.013	0.039	0.694	
		H	0	3	53466.71	49950.29	0.934231599	0.010	0.029	0.523	
				1	59198.48	40825.07	0.689630376	0.007	0.021	0.371	
				2	55863.18	29844.87	0.534249393	0.005	0.015	0.274	
			0.1	3	55264.49	29580.88	0.535260164	0.005	0.015	0.274	
				1	54638.76	62636.93	1.146382714	0.012	0.037	0.655	
				2	53829.12	57084.01	1.060467085	0.011	0.034	0.602	
			0.2	3	53396.47	60647.56	1.135797179	0.012	0.036	0.649	
				1	53177.67	84742.34	1.593570008	0.017	0.052	0.934	
				2	53656.21	73401.27	1.367992074	0.015	0.044	0.793	
			F	0	3	53658.53	66901.14	1.246794126	0.013	0.040	0.718
					1	60757.04	77351.55	1.273129007	0.011	0.033	0.599
					2	61572.1	42574.34	0.691455058	0.004	0.013	0.227
			G	0.1	3	60810.64	44723.2	0.735450244	0.005	0.014	0.255
					1	61364.54	445090.3	7.253216597	0.082	0.247	4.421
					2	62153.11	422888.2	6.803974894	0.077	0.231	4.134
		0.2		3	62144.08	445740.9	7.172700923	0.081	0.244	4.370	
				1	63179.63	1543206	24.4256891	0.287	0.860	15.398	
				2	60028.79	1559855	25.98511481	0.305	0.916	16.395	
		0		3	61296.16	1533981	25.02572755	0.294	0.881	15.782	
				1	59135.15	36967.02	0.625127695	0.003	0.010	0.185	
				2	62066.04	29113.37	0.469070848	0.002	0.005	0.085	
		0.1		3	59659.38	31018.39	0.51992478	0.002	0.007	0.117	
				1	60585.44	43708.9	0.721442314	0.005	0.014	0.246	
				2	62004.77	33767.88	0.544601327	0.002	0.007	0.133	
		0.2		3	61175.8	31875.82	0.521052769	0.002	0.007	0.118	
				1	60658.79	59906.67	0.987600808	0.008	0.023	0.416	
				2	61538	70592.13	1.147130716	0.010	0.029	0.518	

Table C8. Continued

Date	Days	Iron Oxide	NTA. Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
				3	61869.62	73450.41	1.187180558	0.010	0.030	0.544
		H	0	1	59783.23	58442.63	0.977575651	0.008	0.023	0.410
				2	59557.77	46946.67	0.788254329	0.005	0.016	0.289
				3	58884.05	33369.22	0.566693697	0.003	0.008	0.147
			0.1	1	58230.38	78046.36	1.34030312	0.012	0.036	0.642
				2	61723.37	62287.67	1.009142404	0.008	0.024	0.430
				3	61983.38	58742.86	0.947719534	0.007	0.022	0.391
			0.2	1	62117.25	110489.3	1.778721692	0.017	0.052	0.922
				2	61636.46	86674.64	1.406223524	0.013	0.038	0.684
				3	61244.82	84665.55	1.382411606	0.012	0.037	0.669

Table C9. Raw ICP-MS data for dissolution of ferrihydrite (F), goethite (G), and hematite (H) in the presence of EDTA (0mM, 0.1mM, or 0.2mM) with 0mM bicarbonate.

Date	Days	Iron Oxide	EDTA Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (μM)
5/27/10	0	F	0	1	124519.6	217693.4	1.75	0.033	0.098	1.755
				2	126689.9	136888.7	1.08	0.024	0.071	1.267
				3	127103.1	179256.3	1.41	0.028	0.084	1.508
			0.1	1	119688.3	30258.13	0.25	0.001	0.003	0.050
				2	115522.9	37665.3	0.33	0.002	0.006	0.104
				3	115774.3	144539.1	1.25	0.014	0.043	0.778
			0.2	1	113627.1	91186.77	0.80	0.008	0.025	0.452
				2	116999.2	86033.76	0.74	0.008	0.023	0.403
				3	119095.3	119853.8	1.01	0.011	0.034	0.601
		G	0	1	126497.9	45804.56	0.36	0.014	0.041	0.743
				2	128614.6	36195.24	0.28	0.013	0.038	0.684
				3	129144.7	39906.91	0.31	0.013	0.039	0.704
			0.1	1	132119	46760.22	0.35	0.014	0.041	0.737
				2	132598.8	44700.01	0.34	0.013	0.040	0.724
				3	131762	43763.45	0.33	0.013	0.040	0.721
			0.2	1	130878.8	46702.7	0.36	0.014	0.041	0.739
				2	128376.5	56721.76	0.44	0.015	0.045	0.801
				3	132408	76668.21	0.58	0.017	0.050	0.901
		H	0	1	144824	51312.62	0.35	0.014	0.041	0.737
				2	134087.1	47585.86	0.35	0.014	0.041	0.737
				3	134705.7	40502.49	0.30	0.013	0.039	0.698
			0.1	1	133269.9	54290.89	0.41	0.014	0.043	0.776
				2	134926	53281.45	0.39	0.014	0.043	0.767
				3	109091.4	67753.28	0.62	0.017	0.052	0.932
			0.2	1	133259.5	56569.63	0.42	0.015	0.044	0.788
				2	134811.5	93399.77	0.69	0.018	0.055	0.984
				3	136806.9	67660.02	0.49	0.016	0.047	0.839
6/14/10	18	F	0	1	106111.8	78671.34	0.74	0	0	0
				2	105501.1	43555.16	0.41	0	0	0
				3	106801.5	40715.78	0.38	0	0	0
			0.1	1	111254.4	11060440	99.42	1.418	4.255	76.189
				2	111422.3	11142190	100.00	1.427	4.280	76.641
				3	116072.6	11300750	97.36	1.389	4.166	74.598
			0.2	1	110310.2	22090490	200.26	2.871	8.612	154.191
				2	109683.3	22081210	201.32	2.886	8.657	155.010
				3	108901.1	22010400	202.11	2.897	8.692	155.626
		G	0	1	102959.6	60889.71	0.59	0	0	0
				2	101327.5	48971.57	0.48	0	0	0
				3	102297.1	40242.9	0.39	0	0	0
			0.1	1	111229.7	2346991	21.10	0.291	0.872	15.612

Table C9. Continued

Date	Days	Iron Oxide	EDTA Conc (mM)	Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (μM)	
7/6/10	40	H	0.2	2	112873	2767775	24.52	0.340	1.020	18.258
				3	111835	2488210	22.25	0.307	0.922	16.501
				1	111379.6	3482379	31.27	0.437	1.311	23.475
			2	111447.9	2800950	25.13	0.349	1.046	18.731	
			3	111931.5	2558763	22.86	0.316	0.948	16.973	
			0	1	106637.8	70338.59	0.66	0	0	0
				2	106473.4	62858.06	0.59	0	0	0
				3	105895.9	47235.49	0.45	0	0	0
			0.1	1	113696.4	7746273	68.13	0.968	2.904	51.990
				2	113451.8	8855770	78.06	1.111	3.332	59.669
				3	114008.8	8672962	76.07	1.082	3.247	58.133
			0.2	1	112303.5	8892185	79.18	1.127	3.381	60.537
		2		110026.2	9562115	86.91	1.238	3.715	66.514	
		3		111215.4	9865147	88.70	1.264	3.792	67.903	
		F	0	1	97102.23	50461.17	0.52	0	0	0
				2	96696.11	128453	1.33	0.003	0.008	0.142
				3	95578.72	34655.66	0.36	0	0	0
			0.1	1	95342.54	10366560	108.73	1.388	4.164	74.563
				2	95788.88	10695650	111.66	1.426	4.278	76.592
				3	94586.09	10471310	110.71	1.414	4.241	75.933
			0.2	1	94510.46	20945380	221.62	2.844	8.533	152.788
				2	92965.55	21140290	227.40	2.919	8.757	156.792
				3	93373.8	21002190	224.93	2.887	8.661	155.078
		G	0	1	96027.66	77665.98	0.81	0	0	0
				2	95693.8	31826.2	0.33	0	0	0
				3	96036.46	29683.72	0.31	0	0	0
			0.1	1	96671.38	3718718	38.47	0.482	1.445	25.876
				2	97862.35	4654569	47.56	0.599	1.797	32.178
				3	96287.12	3993950	41.48	0.521	1.562	27.963
			0.2	1	96436.74	5615171	58.23	0.737	2.210	39.568
				2	97938.63	4528243	46.24	0.582	1.746	31.259
				3	95302.16	4251834	44.61	0.561	1.683	30.136
		H	0	1	96843.91	53094.03	0.55	0	0	0
				2	97621.02	48668.32	0.50	0	0	0
				3	99687.11	39546.09	0.40	0	0	0
			0.1	1	97393.09	10640320	109.25	1.395	4.185	74.924
				2	98513.27	10986470	111.52	1.424	4.272	76.498
				3	96347.07	10785370	111.94	1.430	4.289	76.789
			0.2	1	96466.85	16142860	167.34	2.144	6.433	115.176
				2	95677.03	15871550	165.89	2.125	6.376	114.169
				3	96093.01	16501800	171.73	2.201	6.602	118.216
		7/25/10	59	F	0	1	100577.7	125262	1.25	0.008

Table C9. Continued

Date	Days	Iron Oxide	EDTA Conc (mM)	Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (μ M)
8/16/2010	81	G	0.1	2	102595	49324.7	0.48	0	0
				3	100598	46910.1	0.47	0	0
				1	103522	1.2E+07	112.63	1.489	4.467
				2	103883	1.2E+07	113.85	1.505	4.516
				3	103819	1.2E+07	114.07	1.508	4.525
			0.2	1	103723	2.4E+07	227.05	3.011	9.032
				2	102976	2.3E+07	227.90	3.022	9.066
				3	101473	2.3E+07	229.14	3.039	9.116
			0	1	99043	54598.4	0.55	0	0
				2	100550	43382.6	0.43	0	0
				3	100932	50243.5	0.50	0	0
			0.1	1	107926	5045302	46.75	0.613	1.839
				2	108292	6204251	57.29	0.753	2.259
				3	107430	5340783	49.71	0.652	1.957
			0.2	1	104580	7398491	70.74	0.932	2.796
				2	107911	6118236	56.70	0.745	2.236
				3	107863	5808240	53.85	0.707	2.122
		H	0	1	99029	136960	1.38	0.009	0.028
				2	98377	53361.4	0.54	0	0
				3	100656	43810.4	0.44	0	0
			0.1	1	116109	1.2E+07	102.96	1.360	4.081
				2	108743	1.1E+07	102.42	1.353	4.060
				3	107604	1.1E+07	104.16	1.376	4.129
			0.2	1	108155	2E+07	188.05	2.492	7.476
				2	106778	2E+07	188.66	2.500	7.501
				3	107193	2E+07	188.53	2.499	7.496
		F	0	1	48476	21278.5	0.44	0	0.011
				2	48250	14793.5	0.31	0	0.006
				3	48049	33510.6	0.70	0.01	0.02
			0.1	1	51114	5466600	106.95	1.24	3.717
				2	50170	5566497	110.95	1.29	3.857
				3	50479	5501384	108.98	1.26	3.788
			0.2	1	50870	1.1E+07	216.17	2.51	7.518
				2	50309	1.1E+07	221.23	2.56	7.694
				3	45584	1.1E+07	245.07	2.84	8.524
		G	0	1	46716	19699.6	0.42	0	0.01
				2	46336	17077.3	0.37	0	0.008
				3	47310	15037.2	0.32	0	0.007
			0.1	1	48562	3165106	65.18	0.75	2.264
				2	50636	3830406	75.65	0.88	2.628
				3	48264	3400568	70.46	0.82	2.447
			0.2	1	49669	4575956	92.13	1.07	3.202

Table C9. Continued

H	0	2	50409	3747617	74.34	0.86	2.583	46.243
		3	50131	3609276	72.00	0.83	2.501	44.78
		1	47596	36563.6	0.77	0.01	0.022	0.3981
		2	47773	18864	0.39	0	0.009	0.1655
		3	47854	27125.1	0.57	0.01	0.015	0.2726
		1	49972	5555966	111.18	1.29	3.865	69.196
	0.1	2	58898	5467672	92.83	1.08	3.226	57.763
		3	50938	5629962	110.53	1.28	3.842	68.788
		1	49393	1.1E+07	219.65	2.55	7.639	136.78
		2	50363	1.1E+07	219.72	2.55	7.642	136.82
		3	50790	1.1E+07	219.92	2.55	7.649	136.95

Table C10. Raw ICP-MS data for dissolution of ferrihydrite (F), goethite (G), and hematite (H) in the presence of NTA (0mM, 0.1mM, or 0.2mM) with 0mM bicarbonate.

Date	Days	Iron Oxide	NTA Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
5/27/10	0	F	0	1	124519.6	217693.4	1.75	0.033	0.098	1.755
				2	126689.9	136888.7	1.08	0.024	0.071	1.267
				3	127103.1	179256.3	1.41	0.028	0.084	1.508
			0.1	1	136746.1	131612.4	0.96	0.022	0.066	1.181
				2	137289	64737.31	0.47	0.015	0.046	0.823
				3	134930.2	70988.26	0.53	0.016	0.048	0.862
			0.2	1	138023.8	72660.44	0.53	0.016	0.048	0.863
				2	136972.1	88549.61	0.65	0.018	0.053	0.950
				3	139065.3	83397.79	0.60	0.017	0.051	0.916
		G	0	1	126497.9	45804.56	0.36	0.014	0.041	0.743
				2	128614.6	36195.24	0.28	0.013	0.038	0.684
				3	129144.7	39906.91	0.31	0.013	0.039	0.704
			0.1	1	139927.7	44187.21	0.32	0.013	0.040	0.709
				2	139685.1	45810.86	0.33	0.013	0.040	0.718
				3	135170.7	41995.64	0.31	0.013	0.039	0.705
			0.2	1	136229.3	47625.36	0.35	0.014	0.041	0.733
				2	134723.6	45376.86	0.34	0.013	0.040	0.724
				3	136331	73955.92	0.54	0.016	0.049	0.874
		H	0	1	144824	51312.62	0.35	0.014	0.041	0.737
				2	134087.1	47585.86	0.35	0.014	0.041	0.737
				3	134705.7	40502.49	0.30	0.013	0.039	0.698
			0.1	1	138400	41928.61	0.30	0.013	0.039	0.699
				2	135325	43881	0.32	0.013	0.040	0.715
				3	137414	44319.14	0.32	0.013	0.040	0.714
			0.2	1	137128.6	47276.19	0.34	0.014	0.041	0.730
				2	137597.9	51440.94	0.37	0.014	0.042	0.751
				3	137941.7	44528.48	0.32	0.013	0.040	0.714
6/14/10	18	F	0	1	106111.8	78671.34	0.74	0	0	0
				2	105501.1	43555.16	0.41	0	0	0
				3	106801.5	40715.78	0.38	0	0	0
			0.1	1	113867.5	1088099	9.56	0.124	0.373	6.682
				2	112645.5	992154.4	8.81	0.114	0.341	6.104
				3	113126.5	1051526	9.30	0.121	0.362	6.481
			0.2	1	101864.1	3098256	30.42	0.425	1.274	22.817
				2	112047.1	3056652	27.28	0.380	1.139	20.392
				3	114561.6	2947980	25.73	0.357	1.072	19.195
		G	0	1	102959.6	60889.71	0.59	0	0	0
				2	101327.5	48971.57	0.48	0	0	0
				3	102297.1	40242.9	0.39	0	0	0
			0.1	1	109645.8	81430.77	0.74	0	0	0
				2	111326	75092.44	0.67	0	0	0

Table C10. Continued

Date	Days	Iron Oxide	NTA Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
7/6/10	40	H	0.2	3	110051.9	59200.36	0.54	0	0	0
				1	112166.9	285699.8	2.55	0.023	0.070	1.261
				2	112517.1	91675.23	0.81	0	0	0
			0	3	114466.7	76391.14	0.67	0	0	0
				1	106637.8	70338.59	0.66	0	0	0
				2	106473.4	62858.06	0.59	0	0	0
			0.1	3	105895.9	47235.49	0.45	0	0	0
				1	114654.9	122775.8	1.07	0.002	0.007	0.119
				2	114363.3	118546	1.04	0.002	0.005	0.093
			0.2	3	113342.2	121462.6	1.07	0.002	0.007	0.120
				1	114820	139324.8	1.21	0.004	0.013	0.230
				2	114012.9	158367.9	1.39	0.007	0.020	0.365
		F	0	3	111744.3	168468.1	1.51	0.009	0.026	0.457
				1	97102.23	50461.17	0.52	0	0	0
				2	96696.11	128453	1.33	0.003	0.008	0.142
			0.1	3	95578.72	34655.66	0.36	0	0	0
				1	94953.17	717349.1	7.55	0.083	0.249	4.456
				2	97951.23	905527.4	9.24	0.105	0.314	5.627
			0.2	3	97743.85	816311.8	8.35	0.093	0.280	5.008
				1	96180.41	2991481	31.10	0.387	1.160	20.773
				2	96362.75	2964460	30.76	0.382	1.147	20.538
		G	0	3	97592.95	3014820	30.89	0.384	1.152	20.627
				1	96027.66	77665.98	0.81	0	0	0
				2	95693.8	31826.2	0.33	0	0	0
			0.1	3	96036.46	29683.72	0.31	0	0	0
				1	95439.99	61731.96	0.65	0	0	0
				2	98008.88	109215.6	1.11	0	0	0
			0.2	3	97992.44	63485.07	0.65	0	0	0
				1	98357.44	212770.6	2.16	0.013	0.040	0.720
				2	97265.48	81558.58	0.84	0	0	0
		H	0	3	97171.73	72432.08	0.75	0	0	0
				1	96843.91	53094.03	0.55	0	0	0
				2	97621.02	48668.32	0.50	0	0	0
			0.1	3	99687.11	39546.09	0.40	0	0	0
				1	97964.97	139763.8	1.43	0.004	0.012	0.210
				2	96550.66	104932.7	1.09	0	0	0
			0.2	3	100720.1	136930.8	1.36	0.003	0.009	0.163
				1	97664.48	165515.6	1.69	0.007	0.022	0.395
				2	96587.53	170476.4	1.76	0.008	0.025	0.444
		F	0	3	97026.02	182683	1.88	0.010	0.029	0.526
				1	100577.7	125262	1.25	0.008	0.023	0.412
7/25/10	59			2	102595.1	49324.7	0.48	0	0	0

Table C10. Continued

Date	Days	Iron Oxide	NTA Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
8/16/10	81	G		3	100597.5	46910.12	0.47	0	0	0
				0.1	1	107584.7	942007.2	8.76	0.108	0.323
				2	108003.5	968296.8	8.97	0.110	0.331	5.927
				3	107740.5	983668.9	9.13	0.113	0.338	6.045
			0.2	1	108245.1	3068325	28.35	0.368	1.104	19.773
				2	107791.7	2957131	27.43	0.356	1.068	19.121
				3	107863.4	3099620	28.74	0.373	1.120	20.052
			0	1	99042.69	54598.39	0.55	0	0	0
				2	100549.7	43382.55	0.43	0	0	0
				3	100932.4	50243.5	0.50	0	0	0
			0.1	1	104135.8	68972.3	0.66	0	0	0
				2	104685	127893.3	1.22	0.007	0.022	0.395
				3	105618.5	68224.27	0.65	0	0	0
			0.2	1	105097.8	228490.9	2.17	0.020	0.060	1.075
				2	105510.9	89238.38	0.85	0.002	0.007	0.126
				3	106706.4	76550.71	0.72	0.001	0.002	0.034
		H	0	1	99029.39	136959.7	1.38	0.009	0.028	0.510
				2	98377.12	53361.36	0.54	0	0	0
				3	100656.2	43810.43	0.44	0	0	0
			0.1	1	106980.5	142438.3	1.33	0.009	0.026	0.473
				2	107025.2	294203	2.75	0.028	0.083	1.486
				3	107892.3	154067.2	1.43	0.010	0.030	0.542
			0.2	1	107122.4	168972.1	1.58	0.012	0.036	0.649
				2	107075.9	204493.9	1.91	0.017	0.050	0.886
				3	108570.9	177208	1.63	0.013	0.038	0.688
		F	0	1	48475.77	21278.49	0.44	0.004	0.011	0.193
				2	48249.54	14793.54	0.31	0.002	0.006	0.110
				3	48048.71	33510.63	0.70	0.007	0.020	0.354
			0.1	1	55960.63	298316.6	5.330829907	0.060	0.181	3.241
				2	49505.55	306214.6	6.185460014	0.070	0.211	3.774
				3	50043.19	308542.9	6.165532213	0.070	0.210	3.761
			0.2	1	49749.02	1554317	31.24316821	0.361	1.083	19.387
				2	50613.13	1500044	29.63744783	0.342	1.027	18.386
				3	49978.75	1529218	30.59736388	0.353	1.060	18.985
		G	0	1	46716.08	19699.62	0.42	0.003	0.010	0.182
				2	46335.57	17077.26	0.37	0.003	0.008	0.149
				3	47309.76	15037.19	0.32	0.002	0.007	0.117
			0.1	1	50141.48	29711.2	0.592547328	0.005	0.016	0.289
				2	47891.76	66602.29	1.3906837	0.015	0.044	0.786
				3	41896.63	53035.25	1.265859569	0.013	0.040	0.708
			0.2	1	48840.31	111646.2	2.285943721	0.025	0.075	1.344
				2	49527.43	41714.06	0.842241562	0.008	0.025	0.444

Table C10. Continued

Date	Days	Iron Oxide	NTA Conc (mM)		Scandium / 45	Iron / 56	Fe/Sc	Fe Conc. (ppm)	Fe Conc in samp (ppm)	Fe Conc in samp (uM)
				3	48556.25	36940.04	0.760767975	0.007	0.022	0.393
		H	0	1	47596.39	36563.56	0.77	0.007	0.022	0.398
				2	47773.46	18863.96	0.39	0.003	0.009	0.165
				3	47853.91	27125.07	0.57	0.005	0.015	0.273
			0.1	1	49115.82	60221.11	1.226104135	0.013	0.038	0.683
				2	48354.14	982969.6	20.32855098	0.234	0.703	12.586
				3	51186.35	324865.1	6.346713528	0.072	0.216	3.874
			0.2	1	54625.45	79229.4	1.45041185	0.015	0.046	0.823
				2	49073.4	77138.42	1.571898829	0.017	0.050	0.899
				3	49241.67	82151.04	1.668323597	0.018	0.054	0.959

APPENDIX D

INVESTIGATING SIDEROPHORE PRODUCTION BY SULFATE-REDUCING
BACTERIA

CHAPTER 1

INTRODUCTION

Sulfur

Sulfur is among the most abundant elements on the Earth and is very important for organisms due to the fact that it is a key constituent in proteins, vitamins, hormones, and other biochemicals. Sulfur recycles itself by oxidation and reduction reactions like other biogeochemical cycles, which include carbon, nitrogen, and phosphorus to name a few. The sulfur cycle is very complex because sulfur has eight different oxidation states, ranging from -2 (completely reduced) to +6 (completely oxidized); however, the most prevalent in nature are -2 (sulfide and reduced organic sulfur), 0 (elemental sulfur), and +6 (sulfate) (Tang, Baskaran and Nemati, 2009). Under anaerobic conditions, hydrogen sulfide is the most stable form of sulfur; however, it is highly reactive. Sulfate (+6) is the most thermodynamically stable and most abundant in oxygen rich environments. Sulfate is chemically inert, nonvolatile, nontoxic, and widespread in rocks, soils, and water. Reduced sulfur is a key constituent of all living organisms which makes sulfate reduction an important step in the recycling of sulfur (Widdel, 1988). Microorganisms play an important role in sulfate reduction and how they reduce sulfate to sulfide can be divided into two pathways, assimilatory sulfate reduction and dissimilatory sulfate reduction. Assimilatory sulfate reduction occurs when an organism reduces sulfate to sulfide and sulfur is incorporated into its proteins or co-factors. All green plants, fungi, and most bacteria reduce sulfate by assimilatory sulfate reduction. Dissimilatory sulfate reduction

involves uptake of sulfate by microorganisms which acts as an oxidizing agent for the breakdown of organic matter and almost all the sulfur is excreted into the environment as sulfide (Postgate, 1984). Dissimilatory sulfate reducers are often described as sulfate-reducing bacteria which are why they are often associated with the “rotten egg” odor from bioproducted H_2S excreted out into the environment.

Sulfate-Reducing Bacteria

Sulfate-reducing bacteria (SRB) are anaerobic and found ubiquitously in nature. SRB use sulfate as a terminal electron acceptor for organic compound degradation which results in the production of sulfide which at environmental pH values of 6.0-9.0 exists mainly as H_2S and HS^- (Lens et al., 1998). There are three main branches of SRB. The gram positive bacteria which include *Desulfotomaculum* and *Desulfosporosinus*, the thermophilic SRB which include *Thermodesulfobacterium* and *Thermodesulfovibrio*, and the δ -subclass of Proteobacteria, consisting of more than 25 genera which include the *Desulfovibrio* genera, the most studied SRB and the main focus in this paper (Tang et al., 2009). SRB have been studied for over a century and still remain an important organism to study because of their economic effects which include but are not limited to: toxicity and pollution caused by H_2S , corrosion of metals and stonework, food spoilage, and souring of oil fields. SRB are model organisms for biotechnology such as bioremediation of organic compounds, immobilization of toxic metals such as chromate and uranium by reduction, and recovery of precious metals (Barton and Fauque, 2009).

SRB have been found in many different environments mostly where there is an abundant supply of sulfate. They have been detected in acid-mine drainage sites where

the pH can be as low as 2 and in soda lakes where the pH can be as high as 10 (Muyzer and Stams, 2008). They have also been detected or isolated from hydrothermal vents, mud volcanoes, marine sediments, oil fields, rice fields, in human diseases, and even the deep sub-surface (Jeanthon et al., 2002, Stadnitskaia et al., 2005, Musmann et al., 2005, Nilsen et al., 1996, Barton and Fauque, 2009, Kovacik et al., 2006). SRB are also present in many uranium contaminated subsurface sites (Abdelouas et al., 1998, Chang et al., 2001, Suzuki and Suko, 2006). *Desulfovibrio desulfuricans* strain G20 (subsequently referred to as G20) was isolated from an oil well corrosion site (Weimer et al., 1988) and *Desulfovibrio vulgaris* strain Hildenborough (subsequently referred to as *D. vulgaris*) was isolated from clay soil near Hildenborough, Kent, United Kingdom (Postgate, 1984). These two organisms are model SRB due to the fact that their genomes have been sequenced (Heidelberg et al., 2004); (NCBI accession No. NC_007519) and they can be easily cultivated. G20 and *D. vulgaris* are the bacteria used in this project.

SRB can further be divided into two main groups based on their metabolic abilities, those that degrade organic compounds completely to carbon dioxide (examples include *Desulfobacter* and *Desulfococcus*) and those that degrade organic compounds incompletely to acetate; *Desulfovibrio* falls into the latter group, incomplete oxidizers which in some cases are able to grow faster than the complete oxidizers (Thauer, Stackebrandt and Hamilton, 2007, Widdel, 1988). Other examples of incomplete oxidizers, also known as acetate oxidizers, include *Desulfobulbus* and *Desulfobacula* to name a few (Tang et al., 2009). *Desulfovibrio* can utilize many various electron donors for sulfate reduction which include but are not limited to hydrogen, dicarboxylic acids

such as malate and fumarate, alcohols such as ethanol and methanol, lactate, amino acids such as glycine and alanine, and sugars such as fructose and glucose (Postgate, 1984, Rabus, Hansen and Widdel, 2006). Besides sulfate, *Desulfovibrio* and other SRB can utilize other sulfur compounds as electron acceptors and reduce to sulfide such as thiosulfate and sulfite. *Desulfovibrio* has also been reported in using nitrate and nitrite as an electron acceptor and reducing to ammonium (Dalsgaard and Bak, 1994). Surprisingly, oxygen has also been reported to be an electron acceptor for some SRB, including *Desulfovibrio*, since previously SRB were considered strict anaerobes (Dilling and Cypionka, 1990). Other electron acceptors for *Desulfovibrio* include the following: Fe(III), U(VI), selenate, chromate, and arsenate (Lovley et al., 1993b, Lovley and Phillips, 1992, Tucker, Barton and Thomson, 1998, Lovley and Phillips, 1994, Macy et al., 2000). However, not all of these reductions were coupled to growth of the SRB.

Sulfur Metabolism/Dissimilatory Sulfate Reduction

The reduction of sulfate to sulfide is the only reaction involving inorganic compounds that requires ATP in biology (Peck Jr., 1993). Four enzymes are needed in an eight-electron reduction process to reduce sulfate to sulfide. The first step in sulfate reduction is the transport of sulfate into the cell since all enzymatic steps occur in the cytoplasm. In dissimilatory sulfate reduction most of the sulfate uptake in SRB expresses a high affinity for uptake when sulfate concentrations are low and decreases its affinity for uptake at higher concentrations (Hansen, 1994). Sulfate uptake is driven by an ion-gradient with *Desulfovibrio* species (Cypionka, 1987), and freshwater species use protons for seaport of sulfate while marine bacteria use Na^+ ions (Rabus et al., 2006).

Once inside the cytoplasm, sulfate is first activated by an enzyme, ATP sulfurylase, to form adenylyl sulfate (APS) and inorganic pyrophosphate (PPi) which is a reversible reaction and two ATP molecules are consumed. This step is necessary due to the unfavorable redox potential of sulfate/bisulfite (-450mV) (Hansen, 1994). PPi is then hydrolyzed by a second enzyme, pyrophosphate phosphohydrolase, since the formation of PPi is thermodynamically unfavorable. APS is further reduced to sulfite (SO_3^-) or the protonated form, bisulfite (HSO_3^-), and AMP by the third enzyme, APS reductase; however, the electron donor for this reaction remains unknown. SRB contain many types of sulfite reductases, in most *Desulfovibrio* species, the bisulfite reductase is referred to as desulfovirdin. The pathway from bisulfite or sulfite reduction to sulfide is unclear; however, it is known that there is a transfer of six electrons to reduce sulfite (+4) to sulfide (-2) and is catalyzed by sulfite reductase (Rabus et al., 2006). Two pathways have been proposed; one involves reduction of bisulfite in a one six-electron step catalyzed by dissimilatory sulfite reductase (DSR) while the other pathway proposes reduction in three two-electron steps via intermediates, trithionate and thiosulfate, also known as the trithionate pathway (Barton and Fauque, 2009, Thauer et al., 2007).

Cytochrome *c3* was the first *c*-type cytochrome discovered in an anaerobic, nonphotosynthetic bacterium by Postgate (1954) in *D. vulgaris* strain Hildenborough as well as the first isolated electron carrier and was later found to be characteristic of all *Desulfovibrio* species. This was the first discovery of these types of pigments and gave a clue to the respiratory type of metabolism in SRB (Widdel, 1988). Cytochrome *c3* has four heme groups per molecule, reacts with oxygen, and is located in the periplasm (Peck

Jr., 1993). Tetraheme cytochrome *c3* has been shown to function as a sulfur reductase and is an essential cofactor of hydrogenases to reduce low-molecular-mass electron transfer proteins (Odom and Peck, 1984). Hydrogenases are a group of enzymes that catalyze the oxidoreduction of the dihydrogen molecule, and *Desulfovibrio* contains three different classes of hydrogenases (Fauque, Legall and Barton, 1991). Cytochrome *c3* has also been shown to reduce oxygen, although no growth was recorded when *Desulfovibrio* used solely oxygen as an electron acceptor (Postgate, 1984). Cytochrome *c3* has also been shown to reduce other metals such as Fe(III) and U(IV) (Lovley et al., 1993c, Payne et al., 2002).

Many SRB are able to grow on only hydrogen and sulfate as energy substrates and hydrogen is one of the best electron donors which results in electron-transport phosphorylation to replace the two ATP molecules that are needed to activate sulfate. Substrate-level phosphorylation occurs when *Desulfovibrio* utilizes lactate as an energy source, which led Odom and Peck (1981) to propose the hydrogen-cycling model which states that lactate is first converted to acetate, then to carbon dioxide, and finally to hydrogen, which diffuses out of the cell and acts as an electron donor for sulfate reduction. This model remains controversial since it has never been refuted or confirmed convincingly (Muyzer and Stams, 2008).

Environmental Impacts

SRB impact the environment in numerous ways, some being detrimental like pollution of soil and water caused by the bioproduction of H₂S. SRB are one of the main organisms that contribute to microbially induced corrosion or biocorrosion of ferrous

materials which results in financial losses to oil and gas companies in the range of \$100 million US dollars annually which accounts for 15-30% of all corrosion cases (Beech and Sunner, 2007). Biocorrosion is a major problem in marine water where sulfate concentrations are high (Muyzer and Stams, 2008). SRB are also responsible for corrosion of concrete and stonework. Bioproduction of H_2S from SRB is oxidized by sulfur oxidizing bacteria to form sulfuric acid which slowly dissolves stonework and concrete (Barton and Fauque, 2009).

However, SRB can also be beneficial to the environment since they have the ability to utilize complex organic molecules as energy sources which makes them excellent candidates for bioremediation strategies. It is estimated that billions of tons of organic and inorganic wastes are produced annually around the world, including metals such as Pb, Cd, As, and Zn (Barton and Fauque, 2009). SRB produce and secrete bisulfide (HS^-), which is highly reactive and can bind to metal cations of the +2 oxidation state leading to precipitating of highly insoluble metal sulfides, which can then be recovered and reused. Bioremediation with SRB and its bioproduced hydrogen sulfide is relatively inexpensive and thus makes SRB a very important organism. SRB, mostly *Desulfovibrio*, are included in the family of dissimilatory metal-reducing bacteria (DMRB). DMRB reduce toxic heavy metals by a process coupled to electron transport. SRB are model organisms for bioremediation since they are tolerant to various heavy metals and are able to reduce toxic heavy metals such as uranium (Barton and Fauque, 2009). An example of using SRB to in bioremediation applications is the commercial process developed by PAQUES BV, which is used in Balk, The Netherlands. This

process treats groundwater from the Budelco zinc refinery using SRB to precipitate Zn^{2+} as ZnS. Then, in an aerobic phase, excess sulfide is oxidized to elemental sulfur (Hockin and Gadd, 2007). Thus, zinc is recovered from the groundwater by using SRB and the problem of hydrogen sulfide pollution is solved.

Siderophores

Siderophores are defined as low molecular weight (500-1000 Daltons) chelating molecules with a very high affinity for iron, specifically ferric iron, to help facilitate solubilization of iron prior to transport into the cell. Siderophores translate to “iron carriers” in Greek and they are produced by aerobic and facultative anaerobic bacteria and by fungi and typically secreted into the extracellular environment when under low iron stress. Their role is to scavenge iron from the environment and make it more bioavailable for microorganisms.

Iron

Iron is the fourth most abundant element in the earth's crust and is second only to aluminum among the metals. Iron can adopt either of two redox states, Fe(II) or Fe(III), and the determination of the oxidation state is influenced by its environment. Although iron is abundant on Earth, it is considered biologically unavailable since iron exists mostly as highly insoluble Fe(III)-(hydr)oxides in oxygen rich environments at nearly neutral pH. Iron is an essential nutrient and is needed for a variety of functions which include but is not limited to reduction of oxygen for ATP synthesis, formation of heme, DNA synthesis, and respiration. Thus, iron is essential for all microorganisms with the

exception of *Lactobacilli*, which utilizes manganese and cobalt in place of iron (Archibald, 1983), and microorganisms have developed sophisticated strategies to obtain the vital nutrient that is considered biologically unavailable.

Both Fe(II) and Fe(III) form six-coordinate octahedral structures with O, N, or S. All oxygen-ligands have a greater affinity to bind to Fe(III) compared to Fe(II) while all nitrogen-ligands have a greater affinity towards Fe(II). This is due to a low reduction potential when only oxygen is coordinated around iron compared to schemes involving only nitrogen in the coordination sphere around iron resulting in a higher reduction potential (Neilands, 1991).

Since the introduction of oxygen into the atmosphere by photosynthetic organisms, iron has slowly been made less available due to oxidation of the iron on the Earth's surface. Under reducing or anaerobic conditions, Fe(II) dominates and can be taken up readily by microorganisms and diffuses freely through the porins located in the outer membrane of gram-negative bacteria without the help of any iron chelators such as siderophores. Under aerobic conditions, solutions of up to 100 mM Fe(II) can be readily taken up by microorganisms due to it being quite soluble (Neilands, 1991); however, Fe(II) is quickly oxidized to Fe(III) and forms insoluble Fe(III) (hydr)oxides such as ferrihydrite, hematite, and goethite. The solubility constant of these $\text{Fe}(\text{OH})_3$ is 10^{-38} in oxic environments at biological pH which limits the concentration of free ferric iron to 10^{-17} - 10^{-18} M, and microorganisms need between 10^{-8} to 10^{-6} M of iron for optimum growth, making iron biologically unavailable. Fe(II) can also be extremely toxic in aerobic conditions due to its involvement in harmful Fenton type reactions (Touati, 2000)

yielding a highly reactive hydroxyl radical notorious for damaging DNA, which leaves aerobic microbes in an environment where a vital nutrient is unavailable due to being insoluble or otherwise toxic. Microorganisms have developed sophisticated strategies to obtain iron from their environment. These strategies include: the reduction of Fe(III) to Fe(II), sequestering iron by the bioproduction of siderophores to solubilize iron, and active transport of the siderophore-iron complex into the cell, the acquisition of iron directly from iron storage proteins such as heme and transferrin, and abstaining from using iron at such as the case with *Lactobacilli* (Richards, 2007; Guerinot, 1994).

Siderophore Structure/Coordination

More than 500 different siderophores have been described to date, the majority being produced by gram-positive and gram-negative bacteria. Siderophores are highly electronegative and have a high affinity for Fe(III), which has a 1:1 stability constant being above 10^{30} M^{-1} , and preferentially forming a hexacoordinated complex with Fe(III). There are three main classes of siderophores based on the type of functional groups present: hydroxamate such examples include the ferrioxamines, ferrichromes, and coprogen, catecholate which include enterobactin, vibriobactins and yersiniabactin, and the α -hydroxycarboxylic acid group includes pyoverdines, azotobactins and ferribactins. Most siderophores are hexadentate ligands and have a higher affinity for Fe(III) compared to Fe(II), which is an optimal denticity, or number of iron binding functional groups, to satisfy the six coordination sites on Fe(III) in a single molecule. However, tetradentate and bidentate siderophores are also produced by microbes and two or three molecules of the ligand assemble to satisfy the six coordination sites of iron. The

denticity of siderophores play an important role in determining its affinity for iron. Generally, the higher the denticity, the greater the affinity towards iron where hexadentate siderophores have a higher affinity to iron compared to siderophores with a lower number of iron binding functional groups (Boukhalfa and Crumbliss, 2002). Cyclic siderophores such as desferrioxamine E have a higher affinity towards Fe(III) compared to the similar linear siderophore, desferrioxamine B (DFB). Thus, both denticity and ligand architecture plays an important role in siderophore evolution. The majority of siderophores are hexadentate ligands and consists of three asymmetrical bidentate functional units attached to an asymmetrical backbone (Matzanke, 1991), which allows for higher stability constants when bound to ferric iron.

The siderophore-iron complex tends to be cyclic in structure to optimize chelate confirmation. Some examples include the siderophores, enterobactin and ferrichrome, bound to iron. Cyclization enhances stability, improves resistance to degrading enzymes, and may play a role in diffusion-controlled transport across cellular membranes by allowing the surface of the siderophore-iron complex to become unreactive after cyclization (Winkelmann, 2002).

Siderophore Binding to Actinides

Siderophores, although highly specific to Fe(III), have been shown to bind and form stable complexes to other heavy metals and actinides (Brainard et al., 1992, Whisenhunt et al., 1996, Neubauer, Furrer and Schulin, 2002). The siderophore, DFB, is a well studied hydroxamate siderophore that is typically found in soil (Powell et al., 1980). DFB has been shown to bind to many divalent heavy metals such as Cu^{2+} , Zn^{2+} ,

Pb^{2+} , and Cd^{2+} to name a few (Esteves et al., 1995, Neubauer et al., 2002, Hepinstall, Turner and Maurice, 2005, Kiss and Farkas, 1998) and also tetravalent actinides such as Pu(IV) , U(IV) , and Th(IV) (Neu et al., 2000, Whisenhunt et al., 1996) to form stable complexes. Since siderophores are capable of chelating other metals besides iron, they have potential in metal recovery and remediation strategies. However, the siderophores, DFB and enterobactin have been shown to solubilize insoluble actinide oxides, and the ferric-siderophore complex was even more effective in solubilizing actinide oxides. Thus, siderophores have the potential to mobilize actinides in the environment (Brainard et al., 1992).

Siderophore Transport

Siderophores are produced by bacteria and fungi typically under low iron conditions and most are secreted out into the extracellular environment to scavenge iron. However, the siderophores, mycobactins, produced from mycobacteria reside in the cell membrane. Once the siderophore secreted by bacteria has obtained iron either from insoluble hydroxides, iron from the surface, or from other iron complexes such as ferric-citrate or ferric phosphate, they are ready to be taken up by the microbial cell by being transported across the cellular membrane. Since the siderophore-iron complex is too large to diffuse across the cell membrane through the porins, with molecular masses of 700-1000 Da (Braun et al., 1998), organisms have developed sophisticated pathways to allow the siderophore-iron complex to enter the cell when iron starved. In bacteria, all genes involved in this process are negatively regulated by the *fur* (ferric uptake regulation) gene (Hantke, 1981). The intake of siderophore-iron complexes into the cell

is an energy driven process involving receptor proteins to form regulated channels specifically for the type of siderophore present, such as ferric hydroxamates and ferric catecholates. Typically, the siderophore specific outer membrane receptors are only formed under low iron conditions and are not present if iron concentrations are sufficient for growth. Once the siderophore-iron complex is inside the cell, a dechelation process occurs, which allows the iron to be released from the siderophore and used by the organism. Two dechelation processes have been proposed, one is the reduction of Fe(III) bound to the siderophore to Fe(II) causing the siderophore to release iron due to its lower affinity towards Fe(II) and the siderophore is recycled. The other proposed mechanism, the siderophore is not recycled and instead is degraded, which allows Fe(III) to be released (Wandersman and Delepelaire, 2004).

REFERENCES

- Archibald, F. (1983). *Lactobacillus plantarum*, an organism not requiring iron. *FEMS Microbiological Letters* , 19, 29-32.
- Barton, L. L., & Fauque, G. D. (2009). Biochemistry, Physiology and Biotechnology of Sulfate-Reducing Bacteria. *Advances in Applied Microbiology* , 68, 41-98.
- Beech, I. B., & Sunner, J. A. (2007). Sulphate-reducing bacteria and their role in corrosion of ferrous materials. In L. L. Barton, & W. A. Hamilton, *Sulphate-Reducing Bacteria* (pp. 459-482). Cambridge, UK: Cambridge University Press.
- Boukhalfa, H., & Crumbliss, A. L. (2002). Chemical aspects of siderophore mediated iron transport. *BioMetals* , 15, 325-339.
- Chang, Y.-J., Peacock, A. D., Long, P. E., Stephen, J. R., McKinley, J. P., Macnaughton, S. J., et al. (2001). Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. *Applied and Environmental Microbiology* , 67 (7), 3149-3160.
- Cypionka, H. (1987). Uptake of sulfate, sulfite and thiosulfate by proton-anion symport in *Desulfovibrio desulfuricans*. *Archives of Microbiology* , 148, 144-149.
- Dalsgaard, T., & Bak, T. (1994). Nitrate reduction in a sulfate-reducing bacterium, *desulfovibrio desulfuricans*, isolated from rice paddy soil- soil inhibition, kinetics, and regulation. *Applied and Environmental Microbiology* , 60, 291-297.
- Dilling, W., & Cypionka, H. (1990). Aerobic respiration in sulfate-reducing bacteria. *Fems Microbiology Letters* , 71, 123-127.
- Esteves, M. A., Vaz, M. C., Goncalves, M., Farkas, E., & Santos, M. A. (1995). Siderophore analogs- synthesis and chelating properties of a new macrocyclic trishydroxamate ligand. *Journal of the Chemical Society- Dalton Tractions* , 2565-2573.
- Fauque, G., Legall, J., & Barton, L. L. (1991). Sulfate-reducing and sulfur-reducing bacteria. In J. M. Shively, & L. L. Barton, *Variations in Autotrophic Life* (pp. 271-337). San Diego: Academic Press Inc.
- Hansen, T. A. (1994). Metabolism of sulfate-reducing prokaryotes. *Antonie van Leeuwenhoek* , 66, 165-185.

- Hantke, K. (1981). Regulation of ferric iron transport in *Escherichia coli* K12-isolation of a constitutive mutant. *Molecular & General Genetics*, 182, 288-292.
- Heidelberg, J. F., Seshadri, R., Haveman, S. A., Hemme, C. L., Paulsen, I. T., Kolonay, J. F., et al. (2004). The genome sequence of the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *Nature Biotechnology*, 22 (5).
- Hepinstall, S. E., Turner, B. F., & Maurice, P. A. (2005). Effects of siderophores on Pb and Cd adsorption to kaolinite. *Clay Minerals Society*, 557-563.
- Hockin, S. L., & Gadd, G. M. (2007). Bioremediation of metals and metalloids by precipitation and cellular binding. In L. L. Barton, & W. A. Hamilton, *Sulphate-Reducing Bacteria* (pp. 405-434). Cambridge, UK: Cambridge University Press.
- Jeanthon, C., L'Haridon, S., Cuffe, V., Banta, A., Reysenbach, A.-L., & Prieur, D. (2002). *Thermodesulfobacterium hydrogenophilum* sp. nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a deep-sea hydrothermal vent at Guaymas Basin, and emendation of the genus *Thermodesulfobacterium*. *International Journal of Systematic and Evolutionary Microbiology*, 52, 765-772.
- Kiss, T., & Farkas, E. (1998). Metal-binding ability of desferrioxamine B. *Journal of INclusion Phenomena and Molecular Recognition in Chemistry*, 32, 385-403.
- Kovacik, W. P. (2006). Molecular analysis of deep subsurface Cretaceous rock indicates abundant Fe(III)- and S-reducing bacteria in a sulfate-rich environment. *Environmental Microbiology*, 8, 141-155.
- Lens, P. N., Visser, A., Janssen, A. J., Hulshoff Pol, L. W., & Lettinga, G. (1998). Biotechnological Treatment of Sulfate-Rich Wastewaters. *Critical Reviews in Environmental Science and Technology*, 28 (1), 41-88.
- Macy, J. M., Santini, J. M., Pauling, B. V., O'Neill, A. H., & Sly, L. I. (2000). Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. *Archives of Microbiology*, 173, 49-57.
- Matzanke, B. F. (1991). Structures, coordination chemistry and functions of microbial iron chelates. In G. Winkelmann, *Handbook of Microbial Iron Chelates* (pp. 15-65). CRC Press, Inc.
- Mubmann, M., Ishii, K., Rabus, R., & Amann, R. (2005). Diversity and Vertical Distribution of Cultured and Uncultured Deltaproteobacteria in an Intertidal Mud Flat of the Wadden Sea. *Environmental Microbiology*, 7 (3), 405-418.

- Muyzer, G., & Stams, A. J. (2008). The Ecology and Biotechnology of Sulphate-Reducing Bacteria. *Nature Reviews Microbiology* , 1-14.
- Neilands, J. B. (1991). A Brief History of Iron Metabolism. *Biology of Metals* , 1-6.
- Neu, M. P., Matonic, J. H., Ruggiero, C. E., & Scott, B. L. (2000). Structural characterization of a plutonium (IV) siderophore complex: Single-crystal structure of Pu-desferrioxamine E. *Angewandte Chemie-International Edition* , 39, 1442.
- Neubauer, U., Furrer, G., & Schulin, R. (2002). Heavy metal sorption on soil minerals affected by the siderophore desferrioxamine B: the role of Fe(III) (hydr)oxides and dissolved Fe(III). *European Journal of Soil Science* , 53, 45-55.
- Nilsen, R. K., Beeder, J., Thostenson, T., & Torsvik, T. (1996). Distribution of thermophilic marine sulfate reducers in North Sea oil field waters and oil reservoirs. *Applied Environmental Microbiology* , 62, 1793-1798.
- Odom, J. M., & Peck, H. D. (1981). Hydrogen cycling as a general mechanism for energy coupling in the sulfate-reducing bacteria, desulfovibrio- sp. *Fems Microbiology Letters* , 12, 47-50.
- Odom, J. M., & Peck, H. D. (1984). Hydrogenase, electron-transfer proteins, and energy coupling in the sulfate-reducing bacteria desulfovibrio. *Fems Microbiology Letters* , 38, 551-592.
- Payne, R. B., Gentry, D. M., Rapp-Giles, B. J., Casalot, L., & Wall, J. D. (2002). Uranium reduction by desulfovibrio desulfuricans strain G20 and a cytochrome c3 mutant. *Applied and Environmental Microbiology* , 68, 3129-3132.
- Peck Jr, H. D. (1993). Bioenergetic strategies of the sulfate-reducing bacteria. In J. M. Odom, & R. Singleton Jr, *The Sulfate-Reducing Bacteria: Contemporary Perspectives* (pp. 41-76). Springer-Verlag New York Inc.
- Postgate, J. R. (1984). *The Sulfate-Reducing Bacteria* (2 ed.). Cambridge: University Press.
- Rabus, R., Hansen, T. A., & Widdel, F. (2006). Dissimilatory sulfate- and sulfur-reducing prokaryotes. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes* (Vol. 2, pp. 659-768). Springer, Berlin: Ecophysiology and Biochemistry.
- Stadnitskaia, A., Muyzer, G., Abbas, B., Coolen, M. J., Hopmans, E. C., Baas, M., et al. (2005). Biomarker and 16S rDNA Evidence for Anaerobic Oxidation of Methane and

Related Carbonate Precipitation in Deep-Sea Mud Volcanoes of the Sorokin Trough, Black Sea. *Marine Geology* , 217, 67-96.

Tang, K., Baskaran, V., & Nemati, M. (2009). Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. *Biochemical Engineering Journal* , 73-94.

Thauer, R. K., Stackebrandt, E., & Hamilton, W. A. (2007). Energy metabolism and phylogenetic diversity of sulphate-reducing bacteria. In L. L. Barton, & W. A. Hamilton, *Sulphate-Reducing Bacteria* (pp. 1-37). Cambridge, UK: Cambridge University Press.

Tucker, M. D., Barton, L. L., & Thomson, B. M. (1998). Reduction of Cr, Mo, Se and U by desulfovibrio desulfuricans immobilized in polyacrylamide gels. *Journal of Industrial Microbiology & Biotechnology* , 20, 13-19.

Weimer, P. J., Van Kavelaar, M. J., Michel, C. B., & Ng, T. K. (1988). Effect of phosphate on the corrosion of carbon steel and on the composition of corrosion products in two-stage continuous cultures of Desulfovibrio desulfuricans. *Applied and Environmental Microbiology* , 54 (2), 386-396.

Whisenhunt, D. W., Neu, M. P., Hou, Z., Xu, J., Hoffman, D. C., & Raymond, K. N. (1996). Specific sequestering agents for the actinides. 29. Stability of the thorium(IV) complexes of desferrioxamine B (DFO) and three octadentate catecholate or hydroxypyridinonate DFO derivatives: DFOMTA, DFOCAMC, and DFO-1,1-HOPO. Comparative stability of the plutonium (IV) DFOMTA complex. *Inorganic Chemistry* , 35, 4128-4136.

Widdel, F. (1988). Microbiology and Ecology of Sulfate- and Sulfur-Reducing Bacteria. In A. J. Zehnder, *Biology of Anaerobic Microorganisms* (pp. 469-586). New York: John Wiley and Sons, Inc.

Winkelmann, G. (2002). Metal Transport. *Biochemical Society* , 30, 691-696.

CHAPTER 2

METHODS

Bacterial Growth and Maintenance*Desulfovibrio vulgaris* Hildenborough

Desulfovibrio vulgaris Hildenborough (*D. vulgaris*), ATCC 29579, were cultured in a defined medium, LS4D medium, with some minor adjustments. The titanium citrate and resazurin solutions were excluded, the medium's pH was adjusted with potassium hydroxide (KOH) (Fisher) instead of sodium hydroxide, and 1,4-Piperazinediethanesulfonic acid (PIPES) (Sigma) was used in place of Piperazine-1,4-Bis(2-ethanesulfonic acid) sesquisodium salt. LS4D medium contains the following: 50mM sodium sulfate (EMD), 60mM sodium lactate (Fisher), 8mM magnesium chloride (Fisher), 20mM ammonium chloride (Sigma), 2.2mM potassium phosphate (Fisher), 0.6mM calcium chloride (Fisher), 1mL/liter of 10x Thauers vitamins, 12.5mL/liter of trace minerals, 30mM PIPES (Sigma), 0.1% resazurin, and 5mL/liter of medium of titanium citrate (Brandis et al., 1981; Huang, 2004).

The 10x Thauers vitamins included the following: 82μM d-Biotin (Sigma), 45μM folic acid (MP), 490μM pyridoxine hydrochloride (Aldrich), 150μM thiamine hydrochloride (Sigma), 130μM riboflavin (Sigma), 410μM nicotinic acid (Sigma), 210μM pantothenic acid (Sigma), 310μM p-aminobenzoic acid (MP), 240μM thiocetic acid (Sigma Aldrich), 14mM choline chloride, and 7.4μM vitamin B12 (US Biochem Corporation). To prepare the vitamins, thiocetic acid was dissolved in 1mL 100% ethanol.

All chemicals were then added to 1L of nanopure water, the pH was adjusted to 7.0 with 1N KOH, and filter sterilized through 0.2 μ m filter system. Then the vitamin solution was degassed with pure nitrogen for about 45 minutes to drive off the oxygen and aliquot into 25mL serum bottles sealed with butyl rubber septa (Bellco Glass, Inc.), capped and crimped with aluminum seals (Fisher), one was stored at 4°C, and all other vials were stored at -20°C (Brandis et al., 1981; Huang, 2004).

The trace minerals included the following: 50mM nitrilotriacetic acid (Acros), 5mM ferrous chloride tetrahydrate (Fisher), 2.5mM manganese chloride tetrahydrate (Fisher), 1.3mM cobalt chloride hexahydrate (Acros), 1.5mM zinc chloride (Fisher), 210 μ M sodium molybdate dihydrate (Fisher), 320 μ M boric acid (Fisher), 380 μ M nickel (II) sulfate hexahydrate, 10 μ M copper (II) chloride dihydrate (Fisher), 30 μ M sodium selenate (Sigma), and 20 μ M sodium tungstate (Fisher). The mineral stock was prepared by adding nitrilotriacetic acid to 1L nanopure water and the pH was adjusted to 6.5 with hydrochloric acid (HCl). All other reagents were added and the pH was readjusted to 6.5 with 5N NaOH. The minerals were filter sterilized through a 0.2 μ m filter system into 500mL serum bottles sealed with butyl rubber septa, capped and crimped with aluminum seals, and stored at 4°C (Brandis et al., 1981; Huang, 2004).

To ensure the medium was made anaerobically, all stock solutions were made by autoclaving nanopure water for 30 minutes on the liquid cycle. The water was degassed with pure nitrogen until the water cooled. Once cooled, chemicals were added and allowed to degas for about 30 minutes longer and autoclaved after distributing to serum bottoms sealed with butyl rubber septa, capped and crimped with aluminum seals on the

liquid cycle for 30 minutes. To make the medium, nanopure water was first autoclaved on liquid cycle for 30 minutes then degassed with pure nitrogen while cooling. Once cooled, the medium ingredients from the stock solutions (except calcium chloride and Thauers vitamins) were added while degassing. The pH was adjusted to 7.2 with KOH after addition of chemicals and medium was transferred to serum bottles to be capped and crimped with aluminum seals. After media was put into serum bottles, the bottles were then autoclaved on liquid cycle for 30 minutes. After the bottles cooled, the calcium chloride and vitamins were added aseptically to each bottle with needles and syringes. Media was stored at room temperature.

D. vulgaris stocks were grown up to log phase, OD₆₀₀ (optical density measured at 600nm) of 0.6 to 0.7, to make stock solutions which were stored at -80°C in a glycerol solution (3mL culture plus 3mL glycerol solution in a 10mL serum bottle). The glycerol solution consisted of the following: 115mL degassed nanopure water, 115mL glycerol, 0.2g cysteine, 0.075g potassium phosphate dibasic, 0.012g potassium phosphate monobasic, 0.025g magnesium sulfate, 0.05g ammonium chloride, 0.05g sodium bicarbonate, 0.2mL vitamin solution (from LS4D), and 2mL trace mineral solution (LS4D). To minimize subculturing, for each experiment 1mL of *D. vulgaris* stock was inoculated into 10mL of fresh LS4D medium. After OD₆₀₀ was about 0.6 to 0.7, this was used as 5% (vol/vol) inocula into fresh LS4D medium. Cultures were grown to log phase, and 10% (vol/vol) inocula were used to start every experiment. Cultures were grown in batch in a 30°C water bath.

Iron limited LS4D medium was made the same as before but modifying the trace minerals. An anaerobic stock of trace minerals were made excluding the ferric chloride tetrahydrate. An anaerobic stock solution of ferric chloride tetrahydrate was also made. The minerals without the iron were added to the medium, 12.5mL per liter of medium, the same as before. Ferric chloride stock was then added to achieve the necessary iron concentration in the medium, the concentrations tested included the following in μM : 62.5, 6.25, 0.625, 0.0625, 6.25nm, 0.625nm, 0, and 0 plus acid washing glassware. All different iron concentration mediums were also tested for siderophore activity after growing for about 4 days. Cultures were centrifuged at 6000rpm for 30 minutes and spent medium was passed through a Bond Elut C₁₈ cartridge to avoid any false positive results with the sulfide produced by the SRB using the methods described in the siderophore detection section below.

Growth curves were done for every different concentration of iron in the LS4D medium and total cell protein was measured using a quantitative colorimetric Coomassie assay method (Pierce, Rockford, IL). Aseptically, 1 mL samples were taken out for every time point, and placed into Axygen microcentrifuge tubes (Fisher) and stored at -20°C until analysis. Once all samples were obtained, all frozen samples were thawed and centrifuged for 10 minutes at 12,000rpm. 0.8mL of supernatant was removed with axygen pipette tips and pellet was re-suspended in 0.8mL of fresh LS4D medium. These were shaken, centrifuged for 10 minutes at 12,000rpm, 0.8mL of supernatant was then removed and 0.8mL of fresh medium was added. Sonication was used for a total of 1.5 minutes for each microcentrifuge tube at 30% amplitude. 0.5mL of the sonicated sample

was then added to 0.5mL of room temperature Coomassie blue (Fisher) and allowed to react for 10min. Optical density was read at 595nm in a 48-well BD Falcon Multiwell Plate and estimated protein (mg/L) in the samples was calculated from a new calibration plot using bovine serum albumin (Fisher) as the standard (0 to 25mg/L) each time samples were analyzed.

Desulfovibrio desulfuricans G20

Desulfovibrio desulfuricans G20 (subsequently referred to as G20) was a gift from Rajesh K. Sani's group, University of North Dakota School of Engineering and Mines (Grand Forks, ND). G20 was maintained in a lactate-C medium modified to metal toxicity medium (MTM). MTM medium consists of the following in grams per liter of medium: sodium lactate, 5.1 (Fisher); sodium sulfate, 2.13 (EMD); calcium chloride dehydrated, 0.06 (Fisher); ammonium chloride, 1 (Sigma); magnesium sulfate, 1; yeast extract, 0.05; tryptone, 0.5; (Fisher) and PIPES, 10.93 (Sigma) (Sani et al., 2001). The medium was made anaerobically by purging with pure nitrogen and using autoclaved degassed nanopure water, the same procedure as making LS4D medium. After medium was aliquot into serum bottles, bottles were sealed with rubber butyl septa, capped and crimped with aluminum seals, and autoclaved. G20 stocks were made from the cultures sent by Sani's group and stored in glycerol solution (the same solution as stated above from *D. vulgaris*) at -80°C. To minimize subculturing during experiments, 1 mL of G20 stock in glycerol was added to 10mL of fresh MTM medium and after about 48 hours, cultures (5% vol/vol) were inoculated into fresh MTM medium. These were allowed to

grow for about 12 hours and 10% (vol/vol) inocula were used for experiments. G20 was grown in batch in a 30°C water bath.

Siderophore Detection

The chrome azurol S (CAS) assay liquid solution included the following: 10mM hexadecyltrimethylammonium bromide (HDTMA) (Sigma), iron solution (1mM ferric chloride hexahydrate in 10mM hydrochloric acid), 2mM aqueous CAS, 0.2M 5-sulfosalicylic acid (Sigma). To make the CAS assay liquid solution, 6mL of 10mM HDTMA was placed into a 100mL volumetric flask and diluted with water. In a separate beaker, 1.5mL of the iron solution and 7.5mL of 2mM CAS solution were stirred together slowly then added to the volumetric flask. In a different beaker, 4.307g of anhydrous piperazine was dissolved in about 10mL of nanopure water. Then 6.25mL of 12M HCl was added to the piperazine solution. This was then added to the volumetric flask and topped off with nanopure water.

Cultures were centrifuged at 6000rpm for 30 minutes. 0.5mL of the supernatant was added to 0.5mL of CAS assay liquid solution and 20μL of 5-sulfosalicylic acid in a disposable cuvette (Fisher). This sat for about 45 minutes to an hour but no more than 6 hours to allow iron exchange from the dye to the siderophore to occur. The optical density was checked at a wavelength of 630nm (Neilands 1986; Payne 1994). To make a calibration plot, desferrioxamine B (DFB) (Sigma) was used as a positive control. Different concentrations of DFB (0μM to 50μM) dissolved in iron-free LS4D medium and purged with compressed air for 4 to 5 hours were checked with the CAS assay. This calibration plot was used to calculate siderophore concentration in the *D. vulgaris*

cultures. In order to use the CAS assay to detect any siderophores, the sulfide in the cultures which is produced by SRBs needed to be disposed of due to it causing a false positive response with the assay. The CAS assay has an iron (III) bound to a dye complex. If siderophores are present, they will bind to iron (III) which releases from the dye and causes a color change. With hydrogen sulfide in the supernatant, iron (III) will bind to the sulfide and causes a false positive response.

The Csáky Assay was used to detect for any hydroxamate siderophores where DFB was used as a positive control. The Csáky assay reagents included the following: sulfanilic acid, 1g sulfanilic acid (Sigma) dissolved by heating in 100mL 30% glacial acetic acid; iodine solution, 1.3g iodine (Aldrich) in 100mL glacial acetic acid; sodium arsenite, 2g sodium arsenite (Sigma) in 100mL nanopure water; sodium acetate, 35g sodium acetate (Fisher) in 100mL nanopure water; α -naphthylamine solution, 3g α -naphthylamine (Sigma) dissolved in 1000mL of 30% acetic acid (stored at 4°C); and 6N sulfuric acid. To test for hydroxamate groups, 1mL of the siderophore solution/supernatant was autoclaved on liquid cycle for 30min. with 1mL of 6N sulfuric acid. Then 3mL of sodium acetate solution was added, followed by 1mL sulfanilic acid solution and 0.5mL iodine solution. This incubated for 3-5min. at room temperature, followed by adding 1mL sodium arsenite solution and mixing. Then 1mL α -naphthylamine solution was added followed by 1.5mL of nanopure water. This then sat for 20-30min. to allow for the color to develop and the absorbance of 1mL was measured at 526nm (Csáky, 1948; Payne, 1994).

The Arnow assay detects for the catecholate functional group in siderophores where 2,3-dihydroxybenzoic acid (2,3-DHB) (Sigma) was used as a positive control. The Arnow assay consists of the following reagents: 0.5N hydrochloric acid; nitrite-molybdate reagent, 10g sodium nitrite (Fisher) and 10g sodium molybdate (Fisher) dissolved in 100mL nanopure water; 1N sodium hydroxide. To test for catecholates, 1mL of 0.5N hydrochloric acid was added to 1mL of siderophore solution/supernatant. 1mL nitrite-molybdate solution was then added followed by 1mL of 1N sodium hydroxide. The OD₅₁₀ was recorded after mixing (Arnow, 1937; Payne, 1994).

Treatment of Sulfide Produced by SRBs

Measuring Sulfide Concentrations

Soluble sulfide in SRB cultures and sulfide-treated cultures were measured spectrophotometrically at 665nm using the methylene blue test (Hach Co., Loveland, CO). This involved diluting 0.5mL of sample with 9.5mL of nanopure water. 0.4mL of sulfide reagent 1 was then added to the solution followed by 0.4mL of sulfide reagent 2. Immediately after mixing, the samples were measured on the spectrophotometer.

Techniques that Failed to Work

A solution of zinc acetate (2.6% and 5.2% were both used) was made as well as 6% NaOH, which then were mixed in a proportion of 5:1. 0.5mL of this solution was added to 0.7mL of culture's (*D. vulgaris* and 2.6% zinc acetate was tested on G20) supernatant in a microcentrifuge tube. This was allowed to react for about 30 minutes. The tubes were centrifuged at 2500rpm for 10 minutes (Gilboa-Garber, 1971). The supernatant was then tested using the CAS assay. A CAS calibration curve was also

made using different concentrations (0-100 μ M) of DFB in LS4D treated with the zinc acetate, both 2.6% and 5.2%, to test the affects of zinc acetate on DFB. Controls consisted of: 100 μ M DFB plus sodium sulfide (50mM and 20mM) in LS4D or MTM, 50 μ M DFB plus sodium sulfide (50mM and 20mM) in LS4D or MTM, and 5 μ M DFB plus sodium sulfide (50mM and 20mM) in LS4D or MTM.

A 0.5mM solution of silver nitrate was made and 100 μ L of this solution was added to 0.9mL of spent medium (both *D. vulgaris* and G20 were used) in microcentrifuge tubes. The tubes were shaken and the silver was allowed to react with sulfide for about 30 minutes. Then these tubes were centrifuged at 2500rpm for 10 minutes. The supernatant was tested for siderophore activity with the CAS assay. A CAS calibration curve was also made using DFB (0-100 μ M) and treated with the silver nitrate as well. Controls used were the same as the ones used with the zinc acetate procedure listed in the preceding paragraph.

Using hydrogen peroxide (H₂O₂) was another method used to try to get rid of sulfide in the SRB cultures. 184 μ L of 30% H₂O₂ was added to 2mL of culture (both *D. vulgaris* and G20) supernatant and this solution was tested for siderophores with the CAS assay. The absorbencies were read after both two hours and 45 minutes. Controls were also tested with H₂O₂ which consisted of 10 μ M of DFB plus spent medium and LS4D or MTM medium.

D. vulgaris cultures were also grown in modified LS4D media by changing the carbon source. One recipe that was used was LS4D with 60mM of sodium pyruvate (Fisher) in place of sodium lactate and sodium sulfate. The other recipe consisted of

LS4D with 60mM sodium pyruvate in place of sodium lactate. Changing the recipes was to help reduce the amount of soluble sulfide in the cultures. *D. vulgaris* was grown in both of these modified LS4D mediums and solid phase extraction (as discussed below) was used on spent medium only from the pyruvate/lactate LS4D. The eluted product was then tested for siderophore activity with the CAS, Arnow, and Csáky assays.

Using Compressed Air to Get Rid of Sulfide

To get rid of the sulfide from cultures an abiotic experiment was first done by purging four 50mL serum bottles with compressed air which contained: 40mL of LS4D medium, 40mL of DFB (15 μ M) in LS4D, 40mL of sodium sulfide (25mM) dissolved in LS4D, and 40mL of DFB (15 μ M) and sodium sulfide (25mM) in LS4D. Each sample's pH was adjusted to about 7 with sulfuric acid. Samples were purged with air for 22 hours total, during which four samples were pulled out of the serum bottles with 3mL syringes. These were then tested using the CAS assay (done in triplicates) as discussed below. After about four hours, samples without DFB showed no false positive with the CAS assay. A CAS calibration curve was also done on different concentration of DFB (0-25 μ M) in LS4D medium to test for any changes in optical response after being purged with compressed air for 4.5 hours.

To test on *D. vulgaris* and G20 cultures, 10mL of 7 and 15 day old cultures' pH was adjusted to about 7 with sulfuric acid and purged with compressed air for 4 to 5 hours. This was then tested for presence of siderophores using the CAS assay method. Controls were treated the same, which consisted of: *D. vulgaris* or G20 culture plus DFB (15 μ M) and LS4D or MTM medium respectively.

Siderophore Isolation

To isolate any siderophores from cultures, *D. vulgaris* and/or G20 cultures were grown in iron-free LS4D (acid washing all glassware) and MTM medium respectively were centrifuged at 6000rpm for 20-30min. The supernatant was retained and used for solid phase extraction. Bond Elut solid phase extraction C₁₈ cartridges (3mL, Varian Inc., Palo Alto, CA) were used and conditioned by first passing 6mL of 100% methanol (Fisher) through the column followed by 6mL of nanopure water. After passing 50mL of spent growth medium through the cartridges, the cartridges were rinsed with 3mL of nanopure water and the siderophores were eluted in 3mL of 100% methanol. To concentrate the crude siderophore extract, air was blown over the extract until only about 5mL remained. The eluent was then tested for siderophore activity with the CAS assay.

Liquid liquid extraction was another method used to isolate siderophores. *D. vulgaris* and/or G20 cultures grown in iron-free (acid washed glassware) LS4D medium and MTM respectively were centrifuged for 6000rpm for 20-30min. The pellet was disposed of and the supernatant was saturated with ammonium sulfate (Fisher) then benzyl alcohol (Fisher) was added at a ratio of 1:5. This mixture was then stirred vigorously overnight. Then this mixture was placed into a separatory funnel and allowed to sit until the layers separated. The aqueous phase (bottom layer) was disposed of and diethyl ether was added to the organic layer in a 4:1 ratio. Nanopure water was then added to this mixture at a ratio of 5:100 and mixed vigorously. The mixture was then placed into the separatory funnel and the bottom layer, which contained the siderophores, was kept. The benzyl alcohol/ether mixture was rinsed two more times with nanopure

water, each time keeping the bottom layer. The crude siderophore extract was concentrated down by compressed air blown over the extract until about 5mL remained and was tested for siderophore activity with the CAS assay.

CHAPTER 3

RESULTS

Growth in Different Iron Concentration Media

Protein growth of *D. vulgaris* in varying concentrations of ferric chloride tetrahydrate was monitored over time as shown in Figure 1. As the iron concentration decreased, the amount of protein also decreased until cell growth reached less than half of the amount that was produced with LS4D, which contains 62.5 μ M ferric chloride tetrahydrate. To help produce maximum siderophore concentrations, the bacteria need to be deprived of iron; therefore, the LS4D medium with no iron added and all acid washed glassware was chosen to check for siderophore production of *D. vulgaris* cultures. Growth for *D. vulgaris* fermenting on pyruvate only is not shown due to how slow growth was, and the growth curve was never completed. A growth curve for G20 in MTM was never completed.

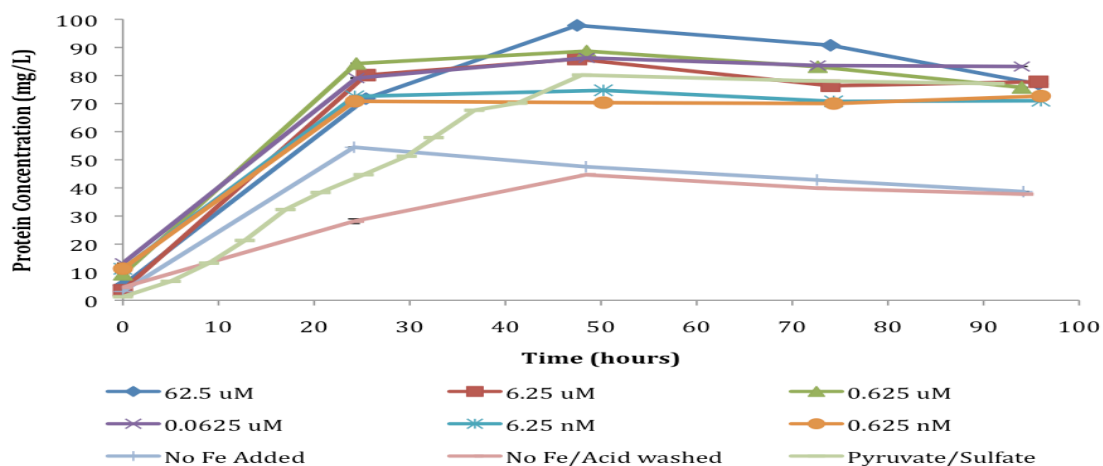


Figure D1. Track of protein concentration in the growth of *D. vulgaris* with varying iron concentrations and also including the growth on Pyruvate and Sulfate LS4D.

Each different iron concentration LS4D medium was also tested for siderophores after 4 days of growing using the CAS, Csáky, and Arnow assays. These assays were tested on both the culture and eluent; however, no siderophores were detected.

Sulfide Treatment

Treating spent medium of *D. vulgaris* and G20 with 2.6% or 5.2% zinc acetate affected the CAS calibration curve after providing treatment to DFB in LS4D medium and not in any type of predicting pattern. One probable cause of this is the ability of DFB to bind to zinc, which affected the optical response, and therefore, this procedure was no longer used as an effective way to precipitate out the sulfide. Treating spent medium with silver nitrate affected the optical response of DFB with the CAS assay also; however, all of these absorbencies could not be read at 630nm.

The use of H_2O_2 to oxidize the sulfide failed to work when using the CAS assay. The length of time the spent medium with H_2O_2 was allowed to react with the CAS assay changed the optical response of this assay. After long periods of time sitting with the CAS solution, the color changed completely, so this method was not used for later experiments.

The only method that did seem to work to get rid of the sulfide and was consistent was purging abiotic controls with compressed air for about 4 to 5 hours after adjusting the pH to 7 as shown in Figure 2. The optical response values did not change after purging for 22 hours in any of the controls. This method was then used on 7 day and 15-day-old cultures.

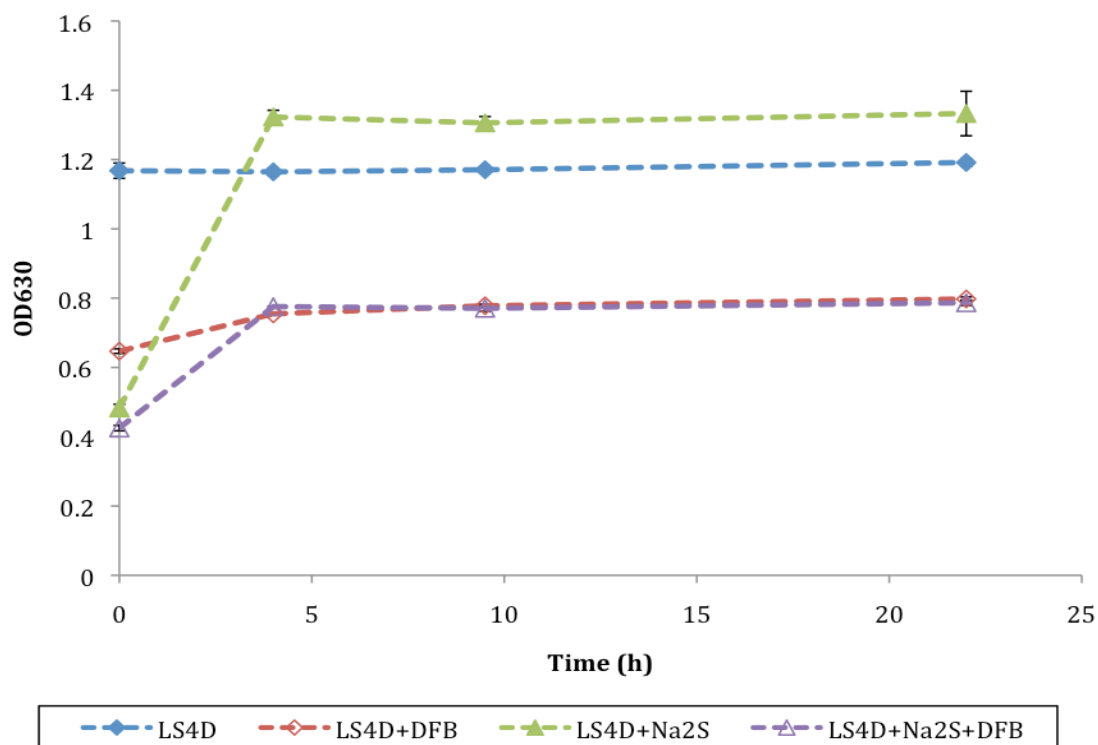


Figure D2. Purging abiotic controls, in triplicates, with compressed air. Concentration of DFB and sodium sulfide (Na_2S) was $15\mu\text{M}$ and 25mM respectively.

Siderophore Detection

7 day old and 15 day old *D. vulgaris* and G20 cultures were purged with air to check for siderophore activity with the CAS assay. In both the 7-day and 15 day G20 cultures, there were no siderophores that were detected (Figure 3 and 4). In both the 7-day and 15 day old *D. vulgaris* cultures about $10\mu\text{M}$ DFB equivalent siderophores were detected as shown in Figures 5 and 6. However, after trying to isolate the siderophores in 12-day-old 800mL cultures using both C_{18} Bond Elut cartridges for solid phase extraction and liquid liquid extraction, no siderophores were detected in the eluent according to the CAS assay.

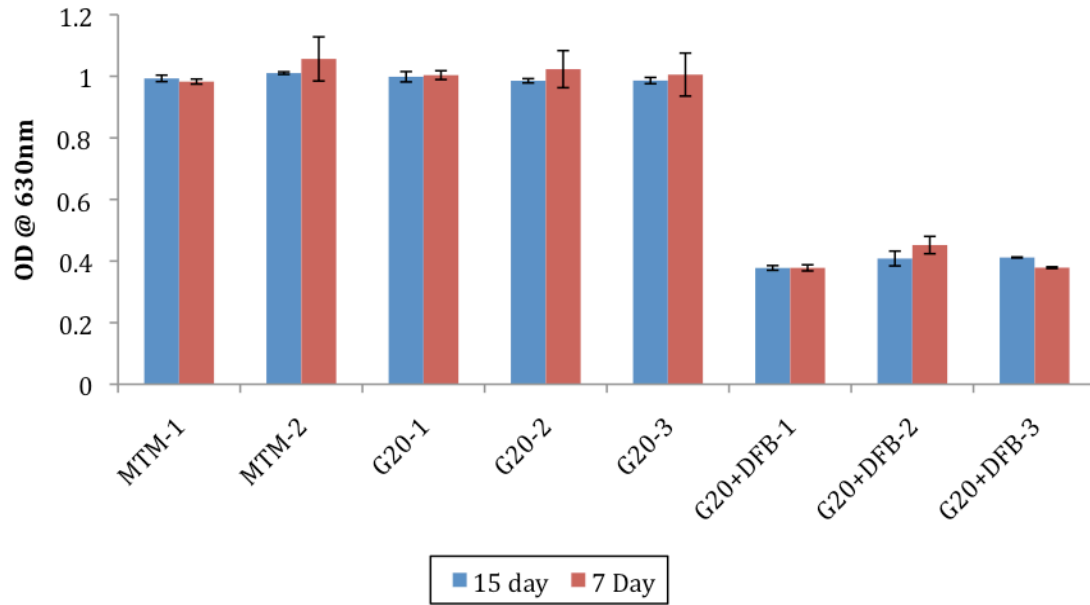


Figure D3. CAS assay (triplicates) results of G20 spent medium after purging with compressed air. Numbers 1-3 are the serum bottle number. DFB concentration used was 15 μ M.

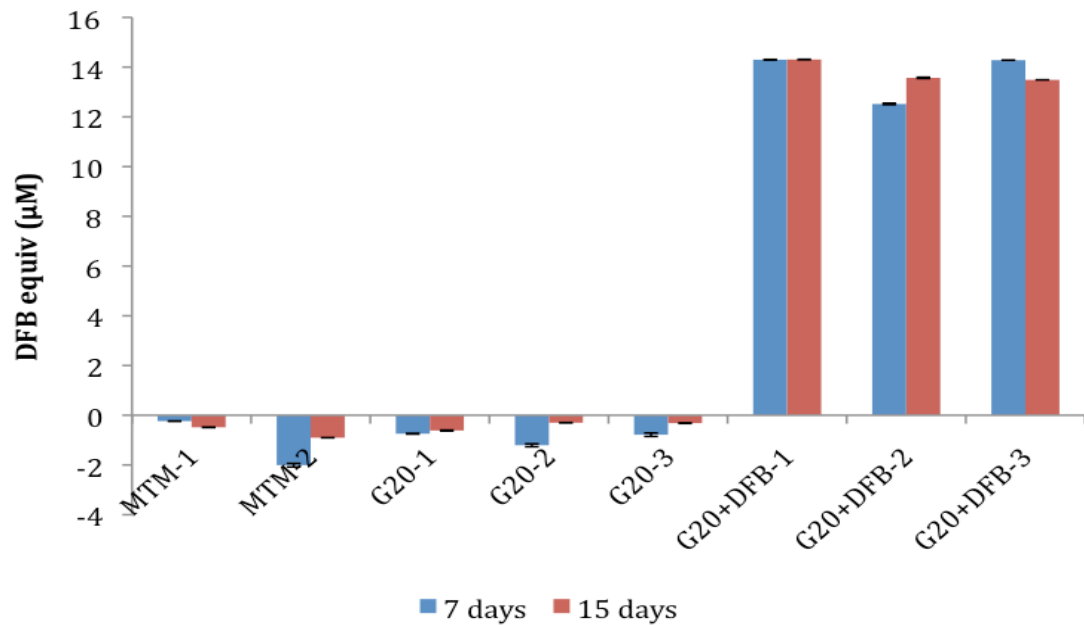


Figure D4. Siderophore production (DFB equivalence) of G20 after being purged with compressed air. DFB concentration used was 15 μ M.

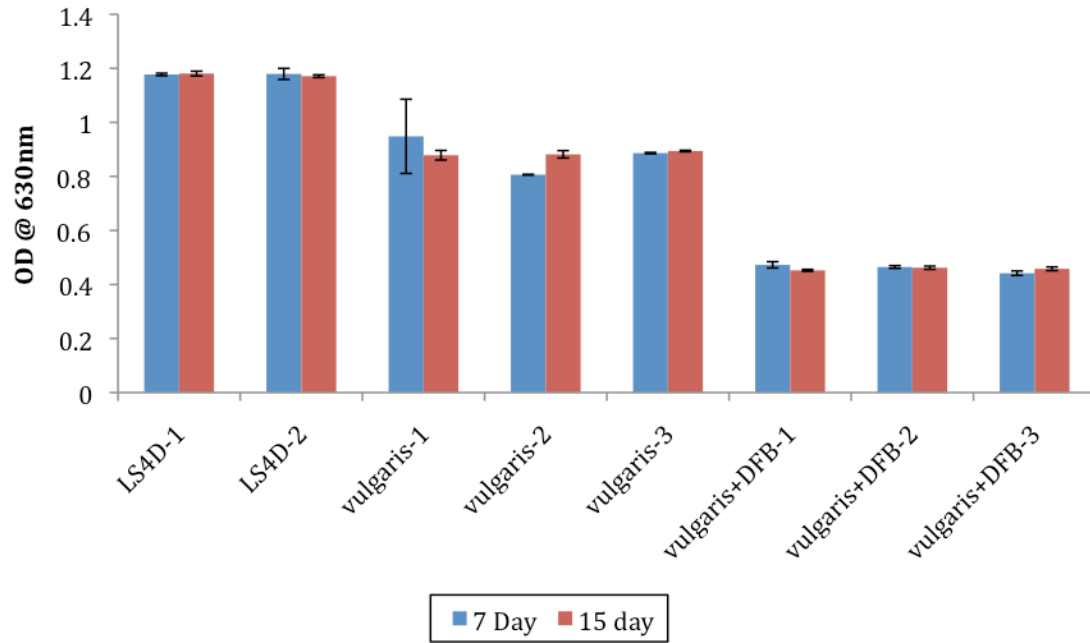


Figure D5. CAS assay (triplicate) results of *D. vulgaris* cultures after being purged with compressed air. Numbers 1-3 represent serum bottle number and DFB concentration used was 15 μ M

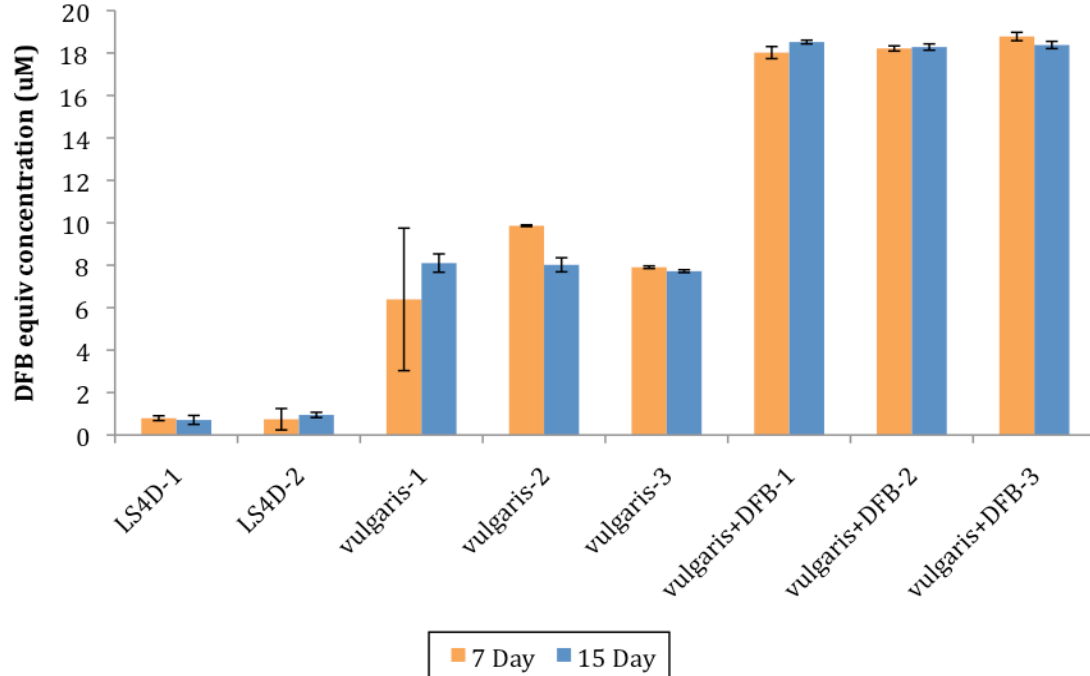


Figure D6. Siderophore production (DFB equivalence) of *D. vulgaris* in 7 and 15 day old cultures after being purged with compressed air. DFB concentration used was 15 μ M.

Discussion

To initiate siderophore production by *D. vulgaris* and G20, cultures were grown under low iron conditions and screened for siderophore production by the CAS, Arnow, and Csáky assays. After initial tests, the bioproducted sulfide from SRB causes a false positive response with the CAS assay. In order to further use the CAS assay, tests were conducted to remove sulfide from cultures without harming any potential siderophores present. Zinc and silver were both used to try to precipitate the sulfide out; however, in controls using DFB, DFB chelated to zinc and silver affecting optical response values. SRB cultures were also treated with hydrogen peroxide, although the hydrogen peroxide was too harmful to the CAS solution. One method that did remove sulfide without affecting DFB controls was the use of purging cultures with compressed air for approximately 5 hours allowing the sulfide to react with hydrogen forming a gas. This method was used on 7 day and 15-day-old SRB cultures.

After purging G20 cultures, no siderophores were detected. However, *D. vulgaris* had approximately 10 μ M DFB equivalence in cultures after 7 days of growth. Solid-phase and liquid-liquid extraction was performed on these cultures; however, no siderophores were detected in the eluent by the CAS assay. HPLC methods were used to further detect any organic molecules in the eluent, and also confirmed no siderophores in the eluent. One possible reason for the negative finding in the eluent could result from solid-phase and/or liquid-liquid extraction not being able to extract the siderophores present in cultures. *D. vulgaris* cultures could also be producing another compound besides siderophores causing a false positive response with the CAS assay explaining

why no siderophores were detected in the eluent. After working on this project for one year, efforts were stopped to isolate siderophores by SRB.

Summary

Siderophores are Fe(III) chelators secreted by many microorganisms to scavenge Fe(III) under conditions where this vital nutrient is biologically unavailable due to being found as solid Fe(III) (hydr)oxides at circumneutral pH. The goal of this project was to investigate siderophore production by SRB, focusing on *D. vulgaris* and *D. desulfuricans* G20, and isolate and characterize any siderophores produced. Sani et al. (2005) have reported uranium reoxidation in the presence of G20 and hematite once cultures became lactate-limited, and one proposed mechanism of oxidation is through the secretion of siderophores. However, this mechanism is unlikely since no siderophores were detected in G20 cultures grown for 15 days under low iron conditions and uranium reoxidation was observed after cultures were grown for 10 days, or after approximately 3 days after the addition of hematite (Sani et al., 2005). Thus, uranium reoxidation by SRB is not initiated by siderophore production but through a different mechanism that is currently unknown.

Siderophores were detected in *D. vulgaris* cultures (approximately 10 μ M in DFB equivalence); however, efforts were unsuccessful in isolating from the medium. Other experiments need to be conducted in concluding *D. vulgaris* does not produce siderophores. Other separation techniques could be used and screened for siderophores using the CAS assay. Older cultures, longer than 15 days, could also be tested as siderophore production occurs when iron is needed. In order to be confident in saying

SRB do not produce siderophores, more research is needed. Other species of SRB need to be tested in their ability to produce siderophores as well as under different growth conditions. Under the growth conditions tested here, we were unsuccessful in isolating siderophores from SRB.

Table D1: Raw data for the Coomassie protein assay to track *D. vulgaris* cell growth (LS4D with 62.5 μ M or 6.25 μ M of iron) at an optical density at 595nm. Eluent was tested for siderophores using the CAS assay at 630nm, Csáky Assay at 526nm, and the Arnow assay at 510nm.

date	time	hours	Fe conc (μ M)		OD52 6	Protein equiv. (mg/L)	OD630 Eluent	OD526 Eluent	OD51 0 Eluent
11/3/08	11:15	0	62.5	1	0.62	5.039215686			
				2	0.648	6.869281046			
				3	0.605	4.058823529			
			6.25	1	0.628	5.562091503			
				2	0.595	3.405228758			
				3	0.57	1.77124183			
11/4/08	12:40	25.4	62.5	1	1.618	70.26797386			
				2	1.556	66.21568627			
				3	1.749	78.83006536			
			6.25	1	1.774	80.46405229			
				2	1.826	83.8627451			
				3	1.707	76.08496732			
11/5/09	10:45	47.5	62.5	1	1.888	87.91503268			
				2	2.013	96.08496732			
				3	2.218	109.4836601			
			6.25	1	1.862	86.21568627			
				2	1.834	84.38562092			
				3	1.873	86.93464052			
11/6/09	13:15	74	62.5	1	1.897	88.50326797			
				2	1.866	86.47712418			
				3	2.031	97.26143791			
			6.25	1	1.603	69.2875817			
				2	1.792	81.64052288			
				3	1.738	78.11111111			
11/7/09	11:00	95.7	62.5	1	1.45	59.2875817	1.241	0.275	0.106
				2	1.846	85.16993464	1.745	0.27	0.159
				3	1.864	86.34640523	0.838	0.259	0.072
			6.25	DFB control 2-3-DHB control LS4D			0.765	0.463	0.035
							0.299	0.262	0.011
							0.621	0.26	0.008
				1	1.726	77.32679739	1.037	0.257	0.063
				2	1.749	78.83006536	0.851	0.259	0.06
				3	1.723	77.13071895	0.911	0.269	0.065
				DFB control 2-3-DHB control LS4D			0.715	0.6	0.06
							0.683	0.255	0.116
							0.769	0.253	0.007

Table D2: Raw data for the Coomassie protein assay to track *D. vulgaris* cell growth at an optical density at 595nm. Eluent was tested for siderophores using the CAS assay at 630nm, Csáky Assay at 526nm, and the Arnow assay at 510nm.

date	time	hours	Feconc		OD526	Protein equiv	OD630	OD526	OD510
11/15/08	12:00	0	0.625	1	0.834	8.329842932			
				2	0.882	10.84293194			
				3	0.84	8.643979058			
			0.0625	1	0.865	9.952879581			
				2	1.025	18.32984293			
				3	0.89	11.2617801			
11/16/08	12:25	24.41	0.625	1	2.144	76.91623037			
				2	2.433	92.04712042			
				3	2.275	83.77486911			
			0.0625	1	2.371	88.80104712			
				2	2.116	75.45026178			
				3	2.069	72.9895288			
11/17/08	12:30	48.5	0.625	1	2.387	89.63874346			
				2	2.408	90.7382199			
				3	2.311	85.65968586			
			0.0625	1	2.484	94.71727749			
				2	2.07	73.04188482			
				3	2.414	91.05235602			
11/18/08	12:40	72.66	0.625	1	2.165	78.01570681			
				2	2.375	89.0104712			
				3	2.247	82.30890052			
			0.0625	1	2.138	76.60209424			
				2	2.268	83.40837696			
				3	2.407	90.68586387			
11/19/08	10:00	94	0.625	1	2.266	83.30366492	0.689	0.101	0.025
				2	1.972	67.91099476	0.774	0.111	0.022
				3	2.127	76.02617801	0.83	0.095	0.027
			0.0625	DFB			0.567	0.107	0.013
				2-3-DHB			0.736	0.107	0.338
				LS4D			0.779	0.106	0.028
				1	2.275	83.77486911	0.879	0.108	0.025
				2	2.272	83.61780105	0.81	0.11	0.048
				3	2.245	82.20418848	1.007	0.108	0.029
				DFB			0.872	0.112	0.056
				2-3-DHB			0.787	0.108	0.341
				LS4D			0.849	0.108	0.018

Table D3: Raw data for the Coomassie protein assay to track *D. vulgaris* cell growth (LS4D with 6.25nM and 0.625nM of iron) at an optical density at 595nm. Eluent was tested for siderophores using the CAS assay at 630nm, Csáky Assay at 526nm, and the Arnow assay at 510nm.

date	time	hours	Fe conc		OD526	Protein Equiv	OD630	OD526	OD510
11/20/08	9:30	0	6.25	1	0.918	12.72774869			
				2	0.865	9.952879581			
				3	0.884	10.94764398			
			0.625	1	0.892	11.36649215			
				2	0.884	10.94764398			
				3	0.895	11.52356021			
			6.25	1	2.044	71.68062827			
				2	2.008	69.79581152			
				3	2.131	76.23560209			
11/21/08	9:45	24.25	0.625	1	2.073	73.19895288			
				2	2.031	71			
				3	1.98	68.32984293			
			6.25	1	2.118	75.55497382			
				2	2.1	74.61256545			
				3	2.088	73.98429319			
			0.625	1	2.021	70.47643979			
				2	1.939	66.18324607			
				3	2.095	74.35078534			
11/23/08	11:50	74.3	6.25	1	2.068	72.93717277			
				2	1.992	68.95811518			
				3	2.021	70.47643979			
			0.625	1	2.048	71.89005236			
				2	1.947	66.60209424			
				3	2.042	71.57591623			
			6.25	1	1.974	68.01570681	0.907	0.108	0.087
				2	2.142	76.81151832	1.034	0.111	0.063
				3	1.978	68.22513089	0.814	0.116	0.079
11/24/08	10:40	96		DFB			0.023	0.138	0.099
				2-3-DHB			0.95	0.118	0.288
				LS4D			0.52	0.12	0.047
			0.625	1	2.165	78.01570681	0.899	0.119	0.089
				2	2.051	72.04712042	0.883	0.113	0.1
				3	1.974	68.01570681	0.751	0.108	0.115
				DFB			0.027	0.14	0.088
				2-3-DHB			0.95	0.118	0.361
				LS4D			0.826	0.116	0.065

Table D4: Raw data for the Coomassie protein assay to track *D. vulgaris* cell growth [LS4D with no added iron and no added iron and acid washed (AW) glassware] at an optical density at 595nm. Eluent was tested for siderophores using the CAS assay at 630nm, Csáky Assay at 526nm, and the Arnow assay at 510nm.

date	time	hours	Fe		OD526	Protein	OD630	OD526	OD510
12/13/08	11:50	0	0	1	0.532	4.2972			
				2	0.498	2.4594			
				3	0.504	2.7837			
				1	0.573	6.5135			
				2	0.564	6.0270			
				3	0.478	1.3783			
				1	1.493	56.243			
				2	1.511	57.216			
				3	1.377	49.972			
12/14/08	12:00	24.17	0	1	1.452	54.027			
				2	1.468	54.891			
				3	----				
				1	1.334	47.648			
				2	1.363	49.216			
				3	1.299	45.756			
				1	1.239	42.513			
				2	1.306	46.135			
				3	1.292	45.378			
12/15/08	12:15	48.42	0	1	1.26	43.648			
				2	1.278	44.621			
				3	1.197	40.243			
				1	1.127	36.459			
				2	1.242	42.675			
				3	1.203	40.567			
				1	1.149	37.648	0.7	0.109	0.055
				2	1.29	45.270	0.748	0.105	0.045
				3	1.065	33.108	0.658	0.117	0.083
12/16/08	12:25	24.12	0	DFB control			0.021	0.122	0.044
				2-3-DHB			0.667	0.107	0.228
				LS4D			0.403	0.105	0.069
				1	1.055	32.567	0.707	0.114	0.088
				2	1.18	39.324	0.818	0.128	0.036
				3	1.219	41.432	0.834	0.126	0.107
				DFB control			0.021	0.104	0.011
				2-3-DHB			0.74	0.111	0.223
				LS4D			0.628	0.113	0.062
				1	1.149	37.648	0.7	0.109	0.055
				2	1.29	45.270	0.748	0.105	0.045
				3	1.065	33.108	0.658	0.117	0.083
				DFB control			0.021	0.122	0.044
				2-3-DHB			0.667	0.107	0.228
				LS4D			0.403	0.105	0.069
12/17/08	10:00	94.12	0	1	1.055	32.567	0.707	0.114	0.088
				2	1.18	39.324	0.818	0.128	0.036
				3	1.219	41.432	0.834	0.126	0.107
				DFB control			0.021	0.104	0.011
				2-3-DHB			0.74	0.111	0.223
				LS4D			0.628	0.113	0.062
				1	1.149	37.648	0.7	0.109	0.055
				2	1.29	45.270	0.748	0.105	0.045
				3	1.065	33.108	0.658	0.117	0.083

Table D5: Raw data to track *D. vulgaris* cell growth (LS4D with Pyruvate and Sulfate (PS)) at an optical density of 600nm and with the Coomassie protein assay at an optical density at 595nm. Eluent was tested for siderophores using the CAS assay at 630nm, Csáky Assay at 526nm, and the Arnow assay at 510nm.

date	time	hours		OD600	OD526	Protein Conc (mg/L)	OD630 Eluent	OD526 Eluent	OD510 Eluent
12/18/08	4:00	0	1	0.025	0.567	1.324324324			
			2	0.024	0.571	1.594594595			
			3	0.025	0.561	0.918918919			
	9:00	5	1	0.051	1.493	9.094594595			
			2	0.083	1.511	5.986486486			
			3	0.05	1.377	5.175675676			
	13:00	9	1	0.093	1.334	14.77027027			
			2	0.084	1.363	12.33783784			
			3	0.089	1.299	12.60810811			
	16:45	12.75	1	0.159	1.26	21.45945946			
			2	0.154	1.278	21.39189189			
			3	0.151	1.197	21.05405405			
	21:00	17	1	0.257	1.149	32.67567568			
			2	0.239	1.29	30.91891892			
			3	0.263	1.065	33.28378378			
12/19/08	0:40	20.67	1	0.351	1.12	38.68918919			
			2	0.341	1.106	37.74324324			
			3	0.359	1.12	38.68918919			
	5:10	25.16	1	0.446	1.216	45.17567568			
			2	0.437	1.193	43.62162162			
			3	0.49	1.218	45.31081081			
	9:40	29.67	1	0.586	1.29	50.17567568			
			2	0.569	1.292	50.31081081			
			3	0.594	1.337	53.35135135			
	12:30	32.5	1	0.641	1.41	58.28378378			
			2	0.603	1.385	56.59459459			
			3	0.617	1.418	58.82432432			
	16:45	36.75	1	0.654	1.516	65.44594595			
			2	0.677	1.563	68.62162162			
			3	0.677	1.566	68.82432432			
	21:15	41.25	1	0.646	1.524	65.98648649			
			2	0.657	1.581	69.83783784			
			3	0.667	1.652	74.63513514			
12/20/08	16:00	48	1		1.723	79.43243243			
			2		1.73	79.90540541			
			3		1.749	81.18918919			
12/21/08	15:00	71	1		1.657	74.97297297			
			2		1.759	81.86486486			
			3		1.697	77.67567568			
12/22/08	11:10	91.16	1		1.668	75.71621622	0.801	0.124	0.086
			2		1.697	77.67567568	0.796	0.103	0.055
			3		1.7	77.87837838	0.693	0.098	0.066
			DFB				0.026	0.148	0.029
			control						
			2-3-DHB				0.888	0.094	0.182
			control						
			PS LS4D				0.864	0.089	0.056

Table D6. Raw data on siderophore detection of 4 day old *D. vulgaris* culture's eluent after liquid liquid extraction using no iron added LS4D or AW, no iron added LS4D using the CAS assay at an optical density of 630nm, Csáky Assay at an optical density of 526nm, and the Arnow assay at an optical density of 510nm.

Wash number	Fe Conc	OD630 Eluent	OD526 Eluent	OD510 Eluent
LS4D 1	0	0.715	0.164	0.01
LS4D 2		0.881	0.143	0.01
LS4D 3		----	----	---
1		0.684	0.156	0.1
2		0.882	0.166	0.011
3		0.919	0.152	0.007
LS4D+DFB 1		0.028	0.311	0.017
LS4D+DFB 2		0.039	0.254	0.009
LS4D+DFB 3		0.042	0.185	0.008
LS4D 1	0 AW	0.792	0.191	0.01
LS4D 2		0.872	0.185	0.006
LS4D 3		0.912	0.205	0.006
1		0.74	0.234	0.027
2		0.898	0.202	0.011
3		0.937	0.199	0.011
LS4D+DFB 1		0.033	0.432	0.015
LS4D+DFB 2		0.04	0.272	0.008
LS4D+DFB 3		0.045	0.268	0.007

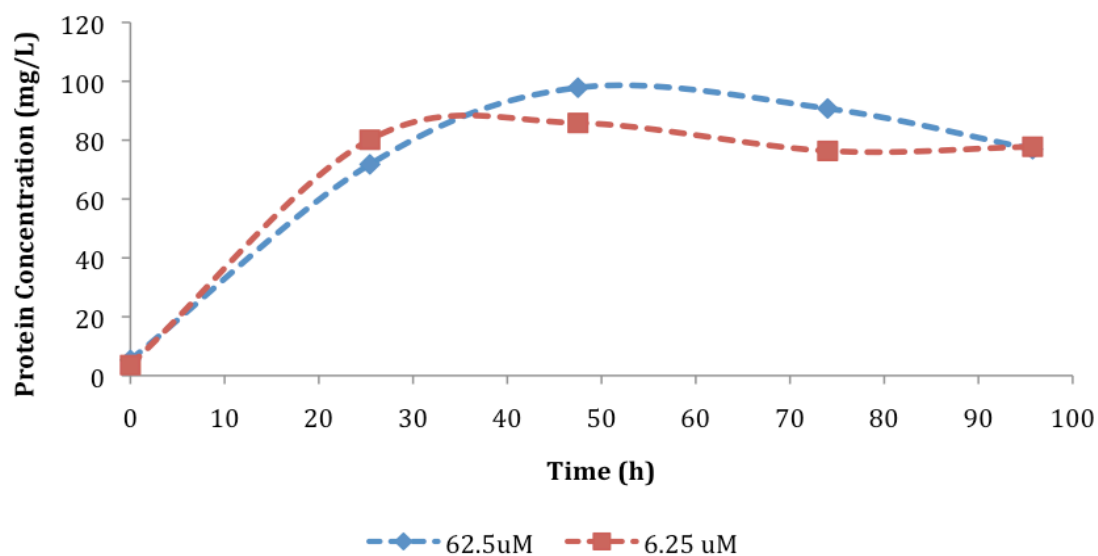


Figure D7. Track of *D. vulgaris* cell growth in protein equivalence (mg/L) with varying iron concentrations.

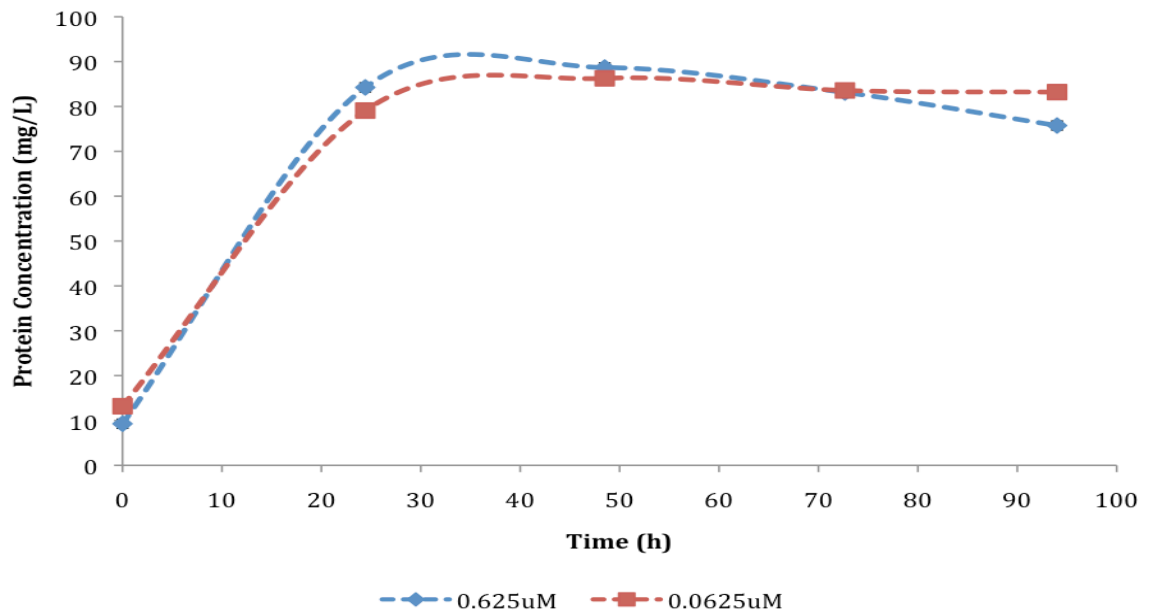


Figure D8. Track of *D. vulgaris* cell growth in protein equivalence (mg/L) with varying iron concentrations.

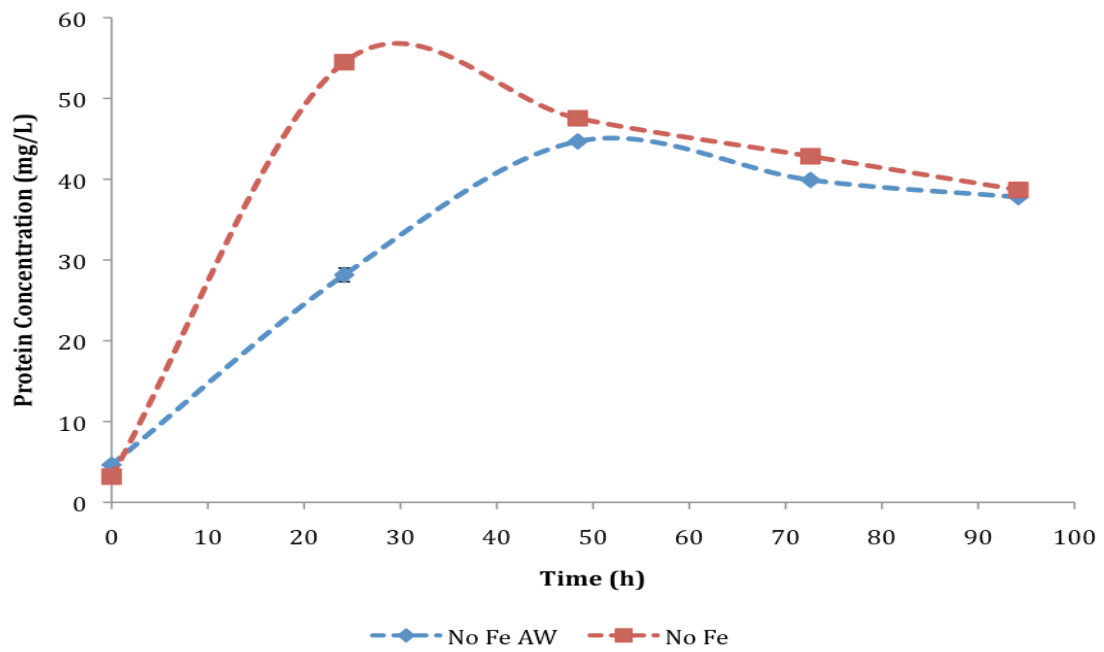


Figure D9. Track of *D. vulgaris* cell growth in protein equivalence (mg/L) with no Fe added LS4D and no Fe added LS4D with acid washed glassware (no Fe/AW LS4D).

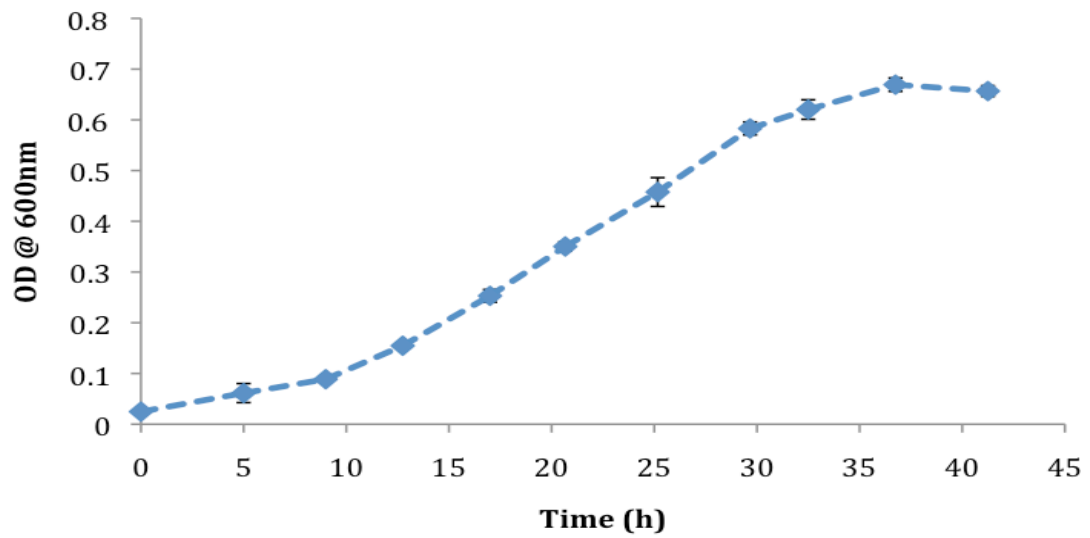


Figure D10. Track of *D. vulgaris* cell growth with optical density in Pyruvate Sulfate (PS) LS4D medium.

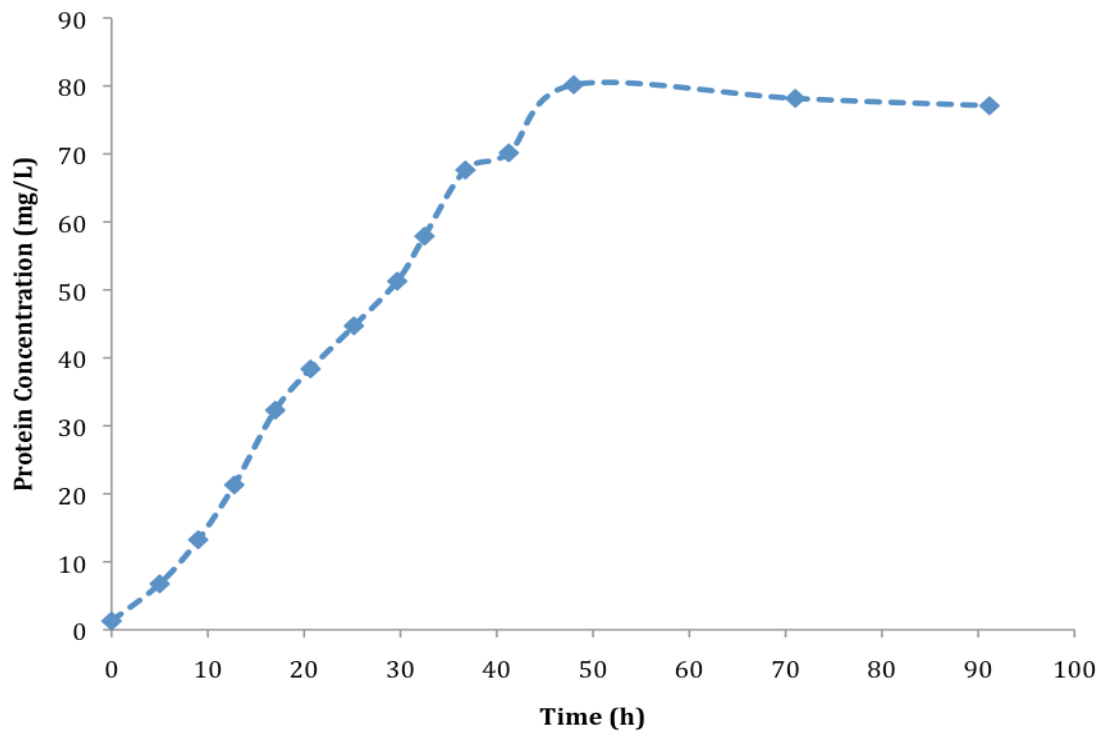


Figure D11. Track of *D. vulgaris* cell growth in protein equivalence (mg/L) in PS LS4D medium.

Table D7. Raw data on the track of siderophore production after sulfide treatment with 2.5% zinc acetate of 7 day old *D. vulgaris* cultures in no Fe LS4D and 4 day old cultures in no Fe/AW LS4D using the CAS, Arnow, and Csáky assays at 630nm, 510nm, and 526nm respectively. Sulfide concentrations were measured with the Hach kit at 665nm.

Date		OD630	OD526	OD510	OD665
2/5/2009	AW 1	0.432	0.089	0.413	1.402
	AW 2	0.369	0.077	1.207	
	AW 3	0.397	0.079	1.388	
	1	0.478	0.075	1.914	1.293
	2	0.468	0.07	1.912	
	3	0.502	0.076	2.58	
	S- AW 1	-----	0.069	0.058	
	S- AW 2	0.281	0.07	0.039	2.634
	S- AW 3	0.303	0.075	0.051	
	S-1	0.411	0.069	0.129	
	S-2	0.236	0.069	0.134	2.874
	S-3	0.283	0.072	0.105	
	AB LS4D	1.061	0.072	0.066	
	No Fe LS4D	1.06	0.069	0.041	
	S- DFB	0.049			
	S- 2,3-DHB	0.607			
	AB/DFB		0.077	0.051	
	No Fe/DFB		0.073	0.015	
	AB/2-3-DHB		0.08	0.345	
	No Fe/ 2-3-DHB		0.076	0.45	

Table D8. Raw data on the track of siderophore production after sulfide treatment with 5.2% zinc acetate of 8 day old *D. vulgaris* cultures in no Fe LS4D and 7 day old cultures in no Fe/AW LS4D using the CAS, Arnow, and Csáky assays at 630nm, 510nm, and 526nm respectively. Sulfide concentrations were measured with the Hach kit at 665nm.

Date		OD630	OD526	OD510	OD665
2/7/2009	AW 1	0.312	0.083		2.739
	AW 2	0.291	0.075		
	AW 3	0.286	0.08	1.225	
	1	0.617	0.09		
	2	0.633	0.079	0.669	
	3	0.629	0.103	1.805	
	S- AW 1	0.924	0.067	0.036	0.044
	S- AW 2	0.913	0.071	0.08	
	S- AW 3	0.929	0.077	0.048	
	S-1	0.911	0.067	0.028	
	S-2	0.911	0.072	0.027	
	S-3	0.905	0.063	0.023	
	AW LS4D	1.059	0.076	0.029	
	No Fe LS4D	1.043	0.065	0.026	
	S-AW	0.834	0.069	0.05	
	S-No Fe	0.841	0.072	0.052	
	S-DFB/AW	0.113	0.085	0.044	
	S- DFB/No Fe	0.833	0.083	0.05	
	S-2,3-DHB/AW	0.836	0.063	0.063	
	S- 2,3-DHB/No Fe	0.824	-----	0.048	
	AB/DFB	0.046	0.093	0.023	
	No Fe/DFB	0.046	0.091	0.019	
	AB/2-3-DHB	1.03	0.078	0.205	
	No Fe/ 2-3-DHB	1.045	0.081	0.272	

Table D9. Raw data on the track of siderophore production after sulfide treatment with 5.2% zinc acetate of 15-day-old *D. vulgaris* cultures in no Fe LS4D using the CAS, Arnow, and Csáky assays at 630nm, 510nm, and 526nm respectively.

Date		OD630	OD526	OD510
2/13/2009	1		0.105	
	2		0.094	
	3		0.088	
	S-1	0.84	0.06	0.022
	S-2	1.047	0.065	0.028
	S-3	0.849	0.063	0.022
	No Fe LS4D	1.049	0.059	0.02
	2,3-DHB	1.067	0.066	0.189
	DFB	0.045	0.076	0.017
	S-No Fe	0.789	0.061	0.042
	S- DFB/No Fe	0.064	0.067	0.032
	S- 2,3-DHB/No Fe	0.794	0.062	0.05

Table D10. Raw data on the track of siderophore production after sulfide treatment with 5.2% zinc acetate of 15-day-old *D. vulgaris* cultures in no Fe/AW LS4D using the CAS and Csáky assays at 630nm and 526nm respectively.

Date		OD630	OD510
2/15/2009	1		
	2		
	3		
	S-1	0.865	0.022
	S-2	0.841	0.019
	S-3	0.83	0.022
	No Fe/AW LS4D	1.105	0.016
	2,3-DHB	1.029	0.189
	DFB	0.047	0.016
	S-AW	0.766	0.063
	S- DFB/AW	0.063	0.031
	S- 2,3-DHB/AW	0.762	0.047

Table D11. Raw data on siderophore production with treatment of sulfide with H₂O₂ measured by the CAS assay at an optical density of 630nm.

Date			OD630 after 2hrs	OD630 after 45min.
2/26/2009	<i>D. vulgaris</i>	1	0.575	0.388
		2	0.592	0.42
		3	0.598	0.418
	<i>D. vulgaris</i> + DFB	1	0.42	0.259
		2	0.431	0.281
		3	0.418	0.241
	LS4D	1	0.662	0.841
		2	0.666	0.904
		3	0.687	0.982

Table D12. Raw data for a CAS calibration curve using 2.6% zinc acetate for the sulfide treatment on controls at an optical density of 630nm.

Date	Conc of DFB (μM)	Conc. Of Na2S (mM)	OD630	OD630 after treatment	
2/12/2009	0		1	0.898	
			2	0.908	
	5		1	0.732	
			2	0.734	
	10		1	0.498	
			2	0.515	
	25		1	0.065	
			2	0.068	
	50		1	0.05	
			2	0.05	
	100		1	0.048	
			2	0.049	
	5		20	1	0.559
			50	1	0.132
	50		20	1	0.082
			50	1	0.12
	100		20	1	0.085
			50	1	0.101

Table D13. Raw data for a CAS calibration curve using silver nitrate for the sulfide treatment on controls at an optical density of 630nm.

Date	Conc of DFB (μ M)	Conc. Of Na2S (mM)	OD630	OD630 after treatment
2/13/2009	0		1	0.916
			2	0.924
	5		1	0.73
			2	0.722
	10		1	0.461
			2	0.468
	25		1	0.064
			2	0.066
	50		1	0.052
			2	0.052
	100		1	0.054
			2	0.05
	5	20	1	1.112
			2	1.151
		50	1	0.259
			2	0.254
	50	20	1	0.099
			2	1.052
		50	1	0.234
			2	0.249
	100	20	1	0.55
			2	0.591
		50	1	0.248
			2	0.255

Table D14. Raw data for a CAS calibration curve using 5.2% zinc acetate for the sulfide treatment on controls at an optical density of 630nm.

Date	Conc of DFB (μM)	Conc. Of Na ₂ S (mM)		OD630	OD630 after treatment
2/13/2009	0		1	0.919	0.792
			2	0.926	0.837
	5		1	0.714	0.76
			2	0.714	0.83
	10		1	0.48	0.681
			2	0.479	0.679
	25		1	0.061	0.381
			2	0.06	0.371
	50		1	0.051	0.062
			2	0.051	0.063
	100		1	0.049	0.063
			2	0.051	0.072
	5	20	1		0.794
			2		0.812
		50	1		0.899
			2		0.895
	50	20	1		0.079
			2		0.072
		50	1		0.89
			2		0.879
	100	20	1		0.094
			2		0.056
		50	1		0.853
			2		0.862
2/15/2009	0		1	0.921	0.828
			2	0.923	0.837
	5		1	0.706	0.747
			2	0.692	0.747
	10		1	0.676	0.738
			2	0.672	0.732
	25		1	0.072	0.367
			2	0.062	0.362
	50		1	0.052	0.059
			2	0.053	0.068
	100		1	0.051	0.059
			2	0.05	0.06
	5	20	1		0.855
			2		0.805
		50	1		0.802
			2		0.862
	50	20	1		0.113
			2		0.107
		50	1		0.092
			2		0.093
	100	20	1		0.058
			2		0.068
		50	1		0.074
			2		0.061

Table D15. Raw data on treatment of sulfide by purging with compressed air and checking for siderophore production using the CAS assay at an optical density of 630nm. Sulfide was measured using the Hach kit at an optical density of 665nm. DFB concentrations were 100 μ M.

Date	Time	hours			OD630	OD665	DFB equiv (μ M)
2/21/2009	12:15	0	<i>D. vulgaris</i>	1	0.443		18.74572127
			<i>D. vulgaris</i> + DFB	1	0.358		20.82396088
			LS4D	1	1.05		3.904645477
	1:00	0.75	<i>D. vulgaris</i>	1	0.256		23.31784841
				2	0.258		23.26894866
			<i>D. vulgaris</i> + DFB	1	0.045		28.47677262
				2	0.052		28.30562347
			LS4D	1	1.156		1.312958435
				2	1.157		1.288508557
	14:15	2	<i>D. vulgaris</i>	1	0.712		12.16870416
				2	0.724		11.87530562
			<i>D. vulgaris</i> + DFB	1	0.047		28.42787286
				2	0.047		28.42787286
			LS4D	1	1.325		-2.819070905
				2	1.315		-2.574572127
	15:15	3	<i>D. vulgaris</i>	1	0.763		10.92176039
				2	0.754		11.14180929
			<i>D. vulgaris</i> + DFB	1	0.045		28.47677262
				2	0.045		28.47677262
			LS4D	1	1.462		-6.168704156
				2	1.438		-5.58190709
	17:00	4.75	<i>D. vulgaris</i>	1	0.695		12.58435208
				2	0.703		12.38875306
			<i>D. vulgaris</i> + DFB	1	0.042		28.55012225
				2	0.042		28.55012225
			LS4D	1	1.488		-6.804400978
				2	1.518		-7.537897311
	19:15	7	<i>D. vulgaris</i>	1	0.703	0.023	12.38875306
				2	0.73		11.72860636
			<i>D. vulgaris</i> + DFB	1	0.042	0.02	28.55012225
				2	0.043		28.52567237
			LS4D	1	1.547		-8.246943765
				2	1.526		-7.733496333

Table D16. Raw data on sulfide treatment on controls in no Fe/AW LS4D with compressed air and measuring siderophore concentration with the CAS assay at an optical density of 630nm. DFB concentration was 10 μ M and Na₂S was 25mM.

Date	Time	hours			OD630	DFB equiv (μ M)
3/7/2009	11:00	0	LS4D	1	1.033	4.320293399
				2	1.039	4.173594132
			DFB	1	0.749	11.26405868
				2	0.758	11.04400978
			Na2S	1	0.303	22.16870416
				2	0.312	21.94865526
			Na2S+DFB	1	0.274	22.87775061
				2	0.292	22.43765281
	15:15	4.25	LS4D	1	1.067	3.488997555
				2	1.063	3.586797066
			DFB	1	0.952	6.300733496
				2	0.949	6.37408313
			Na2S	1	0.275	22.85330073
				2	0.272	22.92665037
			Na2S+DFB	1	0.276	22.82885086
				2	0.271	22.95110024
	19:15	8.25	LS4D	1	1.064	3.562347188
				2	1.228	-0.447432763
			DFB	1	1.009	4.907090465
				2	1.029	4.41809291
			Na2S	1	0.316	21.85085575
				2	0.312	21.94865526
			Na2S+DFB	1	0.306	22.09535452
				2	0.288	22.53545232
	23:15	12.25	LS4D	1	1.055	3.782396088
				2	1.066	3.513447433
			DFB	1	0.996	5.224938875
				2	1.002	5.078239609
			Na2S	1	0.117	26.71638142
				2	0.871	8.281173594
			Na2S+DFB	1	0.062	28.06112469
				2	0.029	28.86797066
3/8/2009	12:15	24.25	LS4D	1	1.079	3.195599022
				2	1.072	3.366748166
			DFB	1	1.042	4.100244499
				2	1.015	4.760391198
			Na2S	1	1.308	-2.403422983
				2	1.329	-2.916870416
			Na2S+DFB	1	0.692	12.65770171
				2	0.829	9.30806846
	15:30	27.5	LS4D	1	1.102	2.633251834
				2	1.094	2.828850856
			DFB	1	1.082	3.122249389
				2	1.08	3.171149144
			Na2S	1	1.541	-8.100244499
				2	1.572	-8.858190709
			Na2S+DFB	1	1.037	4.222493888
				2	1.068	3.464547677

Table D17. Raw data on sulfide treatment on controls (pH adjusted to 7) in no Fe/AW LS4D with compressed air and measuring siderophore concentration with the CAS assay at an optical density of 630nm. DFB concentration was 10 μ M and Na₂S was 25mM.

Date	Time	hours			OD630	DFB equiv (μ M)
3/26/2009	11:15	0	LS4D	1	1.166	1.068459658
				2	1.147	1.533007335
				3	1.191	0.457212714
			DFB	1	0.653	13.61124694
				2	0.64	13.92909535
				3	0.648	13.73349633
			Na2S	1	0.479	17.86552567
				2	0.475	17.96332518
				3	0.495	17.47432763
			Na2S+DFB	1	0.479	17.86552567
				2	0.475	17.96332518
				3	0.495	17.47432763
	15:15	4	LS4D	1	1.155	1.337408313
				2	1.164	1.117359413
				3	1.175	0.848410758
			DFB	1	0.753	11.16625917
				2	0.754	11.14180929
				3	0.757	11.06845966
			Na2S	1	1.315	-2.574572127
				2	1.309	-2.427872861
				3	1.345	-3.30806846
			Na2S+DFB	1	0.776	10.60391198
				2	0.775	10.62836186
				3	0.776	10.60391198
3/27/2009	20:45	9.5	LS4D	1	1.172	0.921760391
				2	1.165	1.092909535
				3	1.175	0.848410758
			DFB	1	0.773	10.67726161
				2	0.779	10.53056235
				3	0.782	10.45721271
			Na2S	1	1.295	-2.085574572
				2	1.296	-2.11002445
				3	1.327	-2.86797066
			Na2S+DFB	1	0.762	10.94621027
				2	0.771	10.72616137
				3	0.779	10.53056235
	9:15	22	LS4D	1	1.179	0.750611247
				2	1.199	0.261613692
				3	1.197	0.310513447
			DFB	1	0.791	10.23716381
				2	0.8	10.01711491
				3	0.802	9.968215159
			Na2S	1	1.292	-2.012224939
				2	1.3	-2.207823961
				3	1.407	-4.82396088
			Na2S+DFB	1	0.785	10.38386308
				2	0.78	10.50611247
				3	0.795	10.1393643

Table D18. Raw data on siderophores production by the CAS assay measuring optical density at 630nm. *D. vulgaris* cultures were 7 days old and treated by adjusting pH to 7 and purging with compressed air for 4.5 hours. DFB concentration was 15 μ M.

Date			OD630	DFB equiv (μ M)
4/6/2009	LS4D	1	1.172	0.921760391
		2	1.18	0.726161369
		3	1.18	0.726161369
	LS4D	1	1.16	1.215158924
		2	1.177	0.799511002
		3	1.201	0.212713936
	<i>D. vulgaris</i>	1	1.107	2.511002445
		2	0.869	8.33007335
		3	0.869	8.33007335
	<i>D. vulgaris</i>	1	0.805	9.894865526
		2	0.808	9.821515892
		3	0.806	9.870415648
	<i>D. vulgaris</i>	1	0.886	7.914425428
		2	0.889	7.841075795
		3	0.884	7.963325183
	<i>D. vulgaris</i> + DFB	1	0.486	17.69437653
		2	0.465	18.20782396
		3	0.467	18.15892421
	<i>D. vulgaris</i> + DFB	1	0.464	18.23227384
		2	0.47	18.08557457
		3	0.46	18.33007335
	<i>D. vulgaris</i> + DFB	1	0.443	18.74572127
		2	0.449	18.599022
		3	0.433	18.99022005

Table D19. Raw data on siderophores production by the CAS assay measuring optical density at 630nm. *D. vulgaris* cultures were 15 days old and treated by adjusting pH to 7 and purging with compressed air for 4.5 hours. DFB concentration was 15 μ M.

Date			OD630	DFB equiv (μ M)
4/14/2009	LS4D	1	1.173	0.897310513
		2	1.19	0.481662592
		3	1.179	0.750611247
	LS4D	1	1.166	1.068459658
		2	1.171	0.946210269
		3	1.176	0.82396088
	<i>D. vulgaris</i>	1	0.887	7.88997555
		2	0.89	7.816625917
		3	0.858	8.599022005
	<i>D. vulgaris</i>	1	0.889	7.841075795
		2	0.89	7.816625917
		3	0.866	8.403422983
	<i>D. vulgaris</i>	1	0.896	7.66992665
		2	0.895	7.694376528
		3	0.891	7.792176039
	<i>D. vulgaris</i> + DFB	1	0.452	18.52567237
		2	0.449	18.599022
		3	0.456	18.42787286
	<i>D. vulgaris</i> + DFB	1	0.455	18.45232274
		2	0.465	18.20782396
		3	0.466	18.18337408
	<i>D. vulgaris</i> + DFB	1	0.45	18.57457213
		2	0.462	18.28117359
		3	0.462	18.28117359

Table D20. Raw data using the CAS assay measuring optical density at 630nm to check for siderophore production on 7 and 12 day old *D. vulgaris* cultures along with eluent from C₁₈ cartridges and from liquid liquid extraction.

Date			OD630	DFB equiv (μM)
4/30/2009 7 day	LS4D	1	1.116	2.290953545
		2	1.15	1.459657702
		3	1.113	2.364303178
	LS4D	1	1.173	0.897310513
		2	1.148	1.508557457
		3	1.149	1.484107579
	<i>D. vulgaris</i>	1	1.039	4.173594132
		2	1.04	4.149144254
		3	1.022	4.589242054
	<i>D. vulgaris</i>	1	0.989	5.39608802
		2	0.993	5.298288509
		3	0.978	5.665036675
	<i>D. vulgaris</i> + DFB	1	0.527	16.69193154
		2	0.538	16.42298289
		3	0.531	16.59413203
	<i>D. vulgaris</i> + DFB	1	0.554	16.03178484
		2	0.56	15.88508557
		3	0.548	16.17848411
5/5/2009 12 day	LS4D	1	1.123	2.119804401
		2	1.137	1.777506112
		3	1.131	1.924205379
	LS4D	1	1.213	-0.080684597
		2	1.228	-0.447432763
		3	1.199	0.261613692
	<i>D. vulgaris</i>	1	0.976	5.71393643
		2	0.973	5.787286064
		3	0.977	5.689486553
	<i>D. vulgaris</i>	1	1.023	4.564792176
		2	1	5.127139364
		3	1.015	4.760391198
	<i>D. vulgaris</i> + DFB	1	0.41	19.55256724
		2	0.419	19.33251834
		3	0.418	19.35696822
	<i>D. vulgaris</i> + DFB	1	0.463	18.25672372
		2	0.465	18.20782396
		3	0.463	18.25672372
5/7/2009 Eluent from C ₁₈	LS4D	1	1.004	
		2	1	
		3	1.023	
	<i>D. vulgaris</i>	1	1.046	
		2	1.054	
		3	1.026	
5/13/2009 Liquid Liquid Extraction	LS4D	1	0.807	
		2	0.827	
		3	0.819	
	<i>D. vulgaris</i>	1	0.871	
		2	0.847	
		3	0.781	

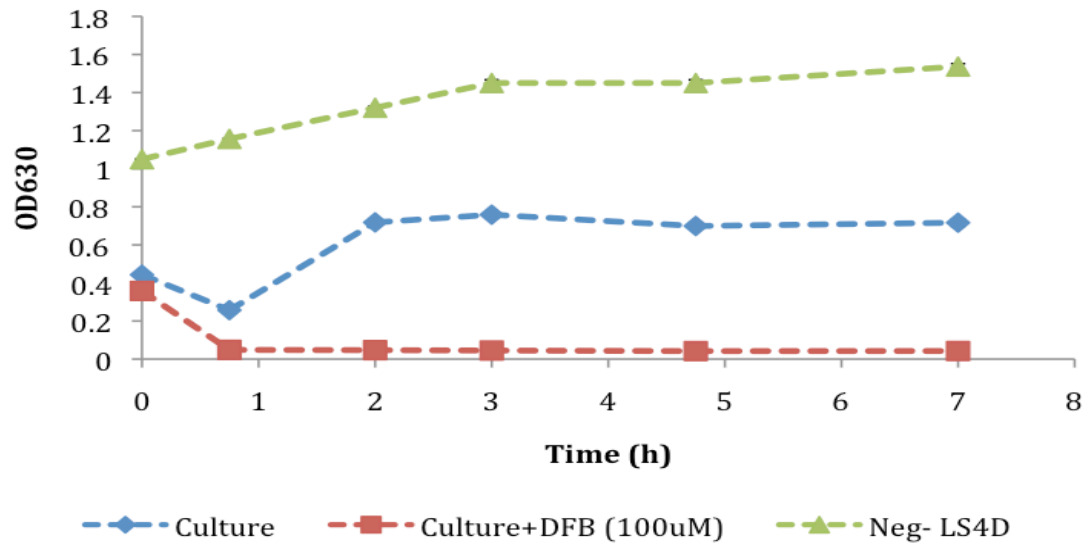


Figure D12. Treatment of sulfide by purging *D. vulgaris* samples with compressed air and checking for siderophore activity using the CAS assay measuring optical density at 630nm.

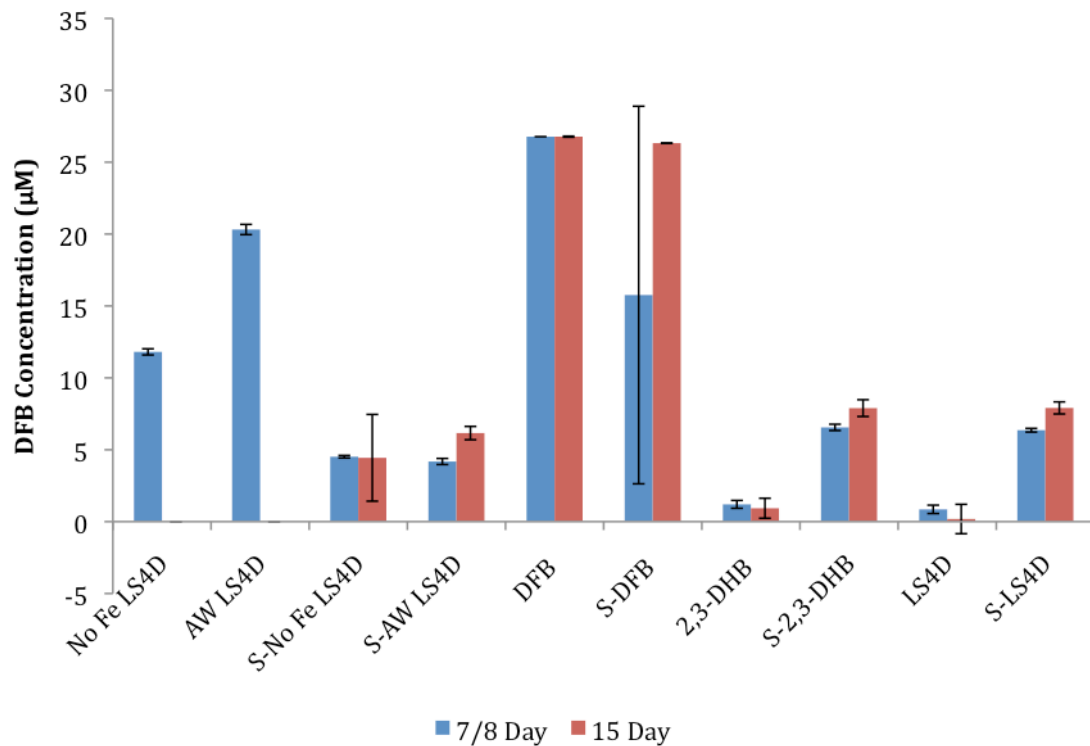


Figure D13. Siderophore production (in DFB equivalence) of 7 or 8 day and 15-day-old *D. vulgaris* cultures using the CAS assay measuring optical density of 630nm. S means treated for sulfide using 5.2% zinc acetate.

Table D21. Raw data on siderophore production by G20 with treatment of sulfide by H_2O_2 with the CAS assay at an optical density of 630nm.

Date			OD630 after 2hrs	OD630 after 45min.
2/26/2009	G20	1	0.872	0.874
		2	0.884	0.891
		3	0.888	0.873
	G20 + DFB	1	0.486	0.53
		2	0.506	0.549
		3	0.506	0.545
	MTM	1	0.77	0.878
		2	0.813	0.881
		3	0.797	0.884

Table D22. Raw data measuring siderophore concentration in G20 cultures using the CAS assay at an optical density of 630nm before and after treatment of sulfide with 2.6% zinc acetate. Sulfide concentrations were also measured after zinc treatment using the Hach kit at an optical density of 665nm.

Date			OD630	OD630 after treatment	OD665
3/10/2009	G20	1	0.361	0.996	1.243
		2	0.352	0.98	0.887
	G20	1	0.359	1.22	
		2	0.34	1.21	
	DFB	1	0.053	0.046	
		2	0.048	0.047	
	2,3-DHB	1	0.958	0.874	
		2	0.972	0.923	
	MTM	1	0.969	0.88	
		2	0.966	0.901	

Table D23. Raw data of siderophore production after purging with compressed air for about 5.5 hours on 7 day and 15-day-old G20 cultures using the CAS assay at an optical density of 630nm.

Date			OD630	DFB equiv (μM)
4/17/2009 7 day	MTM	1	0.977	-0.100961538
		2	0.979	-0.149038462
		3	0.992	-0.461538462
	MTM	1	1.015	-1.014423077
		2	1.139	-3.995192308
		3	1.015	-1.014423077
	G20	1	1.02	-1.134615385
		2	0.998	-0.605769231
		3	0.993	-0.485576923
	G20	1	1.092	-2.865384615
		2	0.995	-0.533653846
		3	0.982	-0.221153846
	G20	1	0.964	0.211538462
		2	0.966	0.163461538
		3	1.086	-2.721153846
	G20 + DFB	1	0.378	14.29807692
		2	0.388	14.05769231
		3	0.368	14.53846154
	G20 + DFB	1	0.448	12.61538462
		2	0.426	13.14423077
		3	0.482	11.79807692
	G20 + DFB	1	0.381	14.22596154
		2	0.379	14.27403846
		3	0.376	14.34615385
4/25/2009 15 day	MTM	1	1	-0.653846154
		2	0.981	-0.197115385
		3	0.998	-0.605769231
	MTM	1	1.015	-1.014423077
		2	1.009	-0.870192308
		3	1.007	-0.822115385
	G20	1	0.98	-0.173076923
		2	1.002	-0.701923077
		3	1.013	-0.966346154
	G20	1	0.992	-0.461538462
		2	0.986	-0.317307692
		3	0.978	-0.125
	G20	1	0.975	-0.052884615
		2	0.995	-0.533653846
		3	0.988	-0.365384615
	G20 + DFB	1	0.369	14.51442308
		2	0.381	14.22596154
		3	0.383	14.17788462
	G20 + DFB	1	0.395	13.88942308
		2	0.436	12.90384615
		3	0.394	13.91346154
	G20 + DFB	1	0.412	13.48076923
		2	0.413	13.45673077
		3	0.41	13.52884615