Competitive Effects of Uranyl-Organic Complexes on U(VI) Reduction by *Shewanella putrefaciens*

Northup

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Competitive Effects of Uranyl-Organic Complexes on U(VI) Reduction by Shewanella putrefaciens

by

Abraham M. Northup

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Science
Department of Geosciences

Western Michigan University
Kalamazoo, Michigan
April 2006
Haas and DiChristina (2002) have demonstrated that Fe(III) reduction by the facultative Fe(III)-reducing bacterium *S. putrefaciens* is mediated by competitive speciation among dissolved organic ligands and functional groups on the cell surface. They also showed that rates of Fe(III) reduction by *S. putrefaciens* correlate with the thermodynamic stability constants of the Fe(III)-organic ligand complexes. *S. putrefaciens* can also use U(VI) as a terminal electron acceptor, coupling U(VI) reduction to growth. In this study, *S. putrefaciens* was incubated in experimental media containing U(VI) in the form of aqueous complexes with a variety of organic ligands that differ significantly in structure and stability with respect to U(VI) chelation. Rates of U(VI) reduction by *S. putrefaciens* vary strongly as a function of U(VI) aqueous speciation. The results of this study indicate that U(VI) reduction under field conditions may be inhibited by the presence of organic chelating ligands.
ACKNOWLEDGMENTS

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I would like to thank the Department of Geosciences staff members, particularly Kathy Wright and Beth Steele for all their help. I would also like to acknowledge Nancy Morgan and Jessica Schoonhoven for their help in setting up experiments as well as sampling and analysis. Noah Ndenga has been a great friend, helping me get back on track when I seem to be lost.
Acknowledgements-Continued

Most importantly, I would like to thank my wife, Shannon, who does not always understand my decisions but she supports me nonetheless.

Abraham M. Northup
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2006
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1. INTRODUCTION

1.1 Background

In modern human culture we rely on the mining of Uranium ores in order to ultimately produce electricity at nuclear power plants as well as to “power” weapons programs. Nuclear power has become a popular alternative in generating electricity as opposed to power plants powered by burning coal or petroleum fuels, which produce air pollution. Incidences at nuclear power facilities such as Three Mile Island, in the U.S., and Chernobyl, in The Former Soviet Union, have essentially halted the construction of new reactors in the U.S. but new reactors are being built abroad. There are approximately 104 nuclear power plants in the U.S. that supply about 20 percent of the nation’s electricity and approximately 439 nuclear power plants worldwide that produce approximately 16 percent of the electricity in the world (NEI 2004). Currently there are 26 nuclear power facilities being constructed in the world (NEI 2005). After WWII the development of the atomic bomb has led many national military weapons programs to develop weapons of mass destruction, which use uranium enriched with respect to $^{235}\text{U}$ as fissible material or more commonly, plutonium, which comes from U powered nuclear reactors.

Depleted uranium (DU), depleted with respect to $^{235}\text{U}$, is used in both civil and military applications. Civil applications of DU include uses as counterweights in commercial aircraft, radiation shields for medical devices, as containers to house and transport radioactive material and as catalysts in specialized chemical reactions primarily related to the oil and gas industries (Betti 2003). In the past,
DU was also used in specialized dental procedures and for coloring glassware and ceramics (Betti 2003). Military applications of DU include uses as armor plating and high-density munitions (Betti 2003 and Giannardi and Dominici 2003).

During the process of extracting and processing the U ore from the earth’s crust large amounts of solid and liquid wastes are produced, such as mine tailings (Abdelouas et al. 1999a). When these wastes are leached of heavy metals, including U(VI) and daughter products of U decay, the leachate infiltrates into the groundwater and flows into the surrounding surface waters contaminating both the ground and surface waters (Abdelouas et al. 1999a).

Much work has gone into possible methods for remediating U contaminated sites (Lovley and Coates, 1997; Abdelouas et al. 1999b; Arey et al. 1999; among others). One type of remediation is the reduction of U(VI) to U(IV) in order to immobilize the uranium in its solid phase. Generally, U(VI) is the soluble form and U(IV) is the insoluble, or solid mineral phase (e.g. Lovley 1993b and Ganesh et al. 1997). When U is reduced, amorphous UO$_2$ is produced which can recrystallize to uraninite (UO$_2$(cr)), which will remain highly insoluble unless re-oxidized to the more soluble U(VI) forms.

It has been demonstrated that U(VI) can be reduced in low temperature geochemical systems by sulfide, organic matter, and Fe(II) (Liger et al. 1999) and Fe$^0$ (Abdelouas et al. 1999b). Previous work has demonstrated that U(VI) can also be reduced microbially, which is the main focus of this study (e.g. Gorby and Lovley 1992; Abdelouas et al. 2000; among others).
1.2 Uranium Chemistry

Uranium is atomic number 92 on the periodic table of elements. Uranium is a naturally occurring radioactive element that has four oxidation states and three isotopes. The oxidation states are $\text{U}^{3+}$, $\text{U}^{4+}$, $\text{UO}_2^+$ (U+5) and $\text{UO}_2^{2+}$ (U+6). The +3 and +5 oxidation states, $\text{U}^{3+}$ and $\text{UO}_2^+$ respectively, are typically unstable relative to the +4 and +6 oxidation states, $\text{U}^{4+}$ and $\text{UO}_2^{2+}$ respectively, are generally more stable. The three natural uranium isotopes are $^{238}\text{U}$, $^{235}\text{U}$ and $^{234}\text{U}$, with $^{238}\text{U}$ being the most abundant and $^{234}\text{U}$ being the least abundant. In nature $^{238}\text{U}$ isotope comprises 99.275% of natural uranium with respect to relative abundance while $^{235}\text{U}$ and $^{234}\text{U}$ comprise 0.719% and 0.0057% respectively. The radioactive decay series of $^{238}\text{U}$ and $^{235}\text{U}$ are represented in Tables 1 and 2 respectively. The $^{234}\text{U}$ isotope decay series is represented within the $^{238}\text{U}$ decay series because it is a daughter product of $^{238}\text{U}$ decay.
<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life</th>
<th>Primary decay mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{238}$U</td>
<td>4.47E9 years</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{234}$Th</td>
<td>24.1 days</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{234}$Pa</td>
<td>6.69 hours</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{234}$U</td>
<td>2.45E5 years</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{230}$Th</td>
<td>7.5E4 years</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{226}$Ra</td>
<td>1,599 years</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{222}$Rn</td>
<td>3,823 days</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{218}$Po</td>
<td>3.04 minutes</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{214}$Pb</td>
<td>26.9 minutes</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{214}$Bi</td>
<td>19.7 minutes</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{214}$Po</td>
<td>1.6E-4 seconds</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{210}$Pb</td>
<td>22.6 years</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{210}$Bi</td>
<td>5.01 days</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{210}$Po</td>
<td>138.4 days</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{206}$Pb</td>
<td>Stable</td>
<td>Stable</td>
</tr>
</tbody>
</table>

Table 1: $^{238}$U and $^{234}$U decay series (Modified from Bourdon et al. 2003)

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life</th>
<th>Primary decay mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{235}$U</td>
<td>7.04E8 years</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{231}$Th</td>
<td>1.06 days</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{231}$Pa</td>
<td>3.28E4 years</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{227}$Ac</td>
<td>21.8 years</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{227}$Th</td>
<td>18.7 days</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{223}$Ra</td>
<td>11.4 days</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{219}$Rn</td>
<td>3.96 seconds</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{215}$Po</td>
<td>1.8E-3 seconds</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{211}$Pb</td>
<td>36.1 minutes</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{211}$Bi</td>
<td>2.14 minutes</td>
<td>$\alpha$ Transmission</td>
</tr>
<tr>
<td>$^{207}$Ti</td>
<td>4.77 minutes</td>
<td>$\beta$ Transmission</td>
</tr>
<tr>
<td>$^{207}$Pb</td>
<td>Stable</td>
<td>Stable</td>
</tr>
</tbody>
</table>

Table 2: $^{235}$U decay series (Modified from Bourdon et al. 2003)

Uranium can exist either as a solid mineral, or as a dissolved species, either complexed or uncomplexed. Most uranium bearing minerals contain U in either the +4 or +6 oxidation state, in a few cases a mixed valence state with both +4 and +6 oxidation states is present, and in at least one case U is in the +5
oxidation state (Burns 1999). The most important uranium-bearing mineral to industrial uses of U is uraninite (UO$_{2+X}$) because it is the primary constituent of uranium bearing ores (Burns 1999; Finch and Murakami 1999). In uraninite U exists in the reduced +4 oxidation state. Pure uraninite (UO$_2$) does not exist in nature because it is always at least partly oxidized, resulting in UO$_{2+X}$, where $X < 0.25$-0.3 (Finch and Murakami 1999). Uraninite commonly occurs in massive forms, which are referred to as pitchblende (Perkins 1998). Uraninite can also occur in individual crystals, which are quite rare, and will display either a cubic or octahedral form or combinations of the two forms (Perkins 1998). Uranium bearing minerals, such as uraninite, where uranium is present as U(IV), tend to be sparingly soluble (Murphy and Shock 1999). Uranium that exists in the +6 oxidation state, such as schoepite ((UO$_2$)$_6$O$_2$(OH)$_{12}$(H$_2$O)$_{12}$), are fairly soluble as dissolved uranyl ion (UO$_{2}^{2+}$) and uranyl complexes may accumulate in solution (Finch and Murakami 1999; Murphy and Shock 1999). Zielinski and Meier (1988) indicate that hexavalent uranium can exist in peat bogs even when conditions are primarily reducing, likely due to uranyl-carbonate complexes. It has been suggested that U(VI) reduction and immobilization by microbes is a controlling factor in the uranium cycle (Barnes and Cochran 1993; Lovley et al. 1993; McKee and Todd 1993).
1.3 Sources of Contamination

1.3.1 Mining and Milling

In order to use uranium for any purpose it first needs to be mined, which is a major source of contamination. Mining of uranium is commonly performed using open pit and deep shaft mining techniques (US NRC 2005). Solution extraction mining is an alternative technique for extracting uranium from low-grade deposits in the subsurface (UIC 2003a; US NRC 2005). Solution extraction is an in situ method in which solutions are pumped into uranium bearing ores to dissolve uranium, which is then extracted from the subsurface (UIC 2003a; US NRC 2005).

Uranium contamination associated with traditional mining techniques, open pit and deep shaft, is generally correlated to the waste material, referred to as tailings, produced from the mining operations. Mine tailings are produced during the mining and milling operations at a mining facility and commonly discarded near the site. After the milling operation the final product is $\text{U}_3\text{O}_8$, which is often referred to as “Yellow Cake” due to its yellow color. The tailings piles generally consist of wallrock and gangue minerals, which contain uranium and daughter products of uranium decay as well as other heavy metals. Sulfide minerals such as pyrite are commonly associated with uranium ores and when tailings are generated, sulfide minerals are likely to be present.

Sulfide minerals are removed from the subsurface along with the ore and deposited in the tailings piles along with the other waste material. Once the tailings are exposed to oxygen the sulfide minerals start to oxidize. Ferrous iron
also oxidizes to ferric iron in the presence of oxygen and subsequently the ferric iron acts as an electron acceptor to oxidize sulfide minerals even more efficiently than oxygen. During the oxidation process of sulfide minerals excess protons in the form of $H^+$ are produced which causes the pH to decrease. It has also been shown that *Thiobacillus ferrooxidans* can increase the oxidation of pyrite by 5 or 6 orders of magnitude greatly increasing the rate of oxidation (Lovley 1993a; Abdelouas et al. 1999a). Equations 1-5 illustrate the oxidation cycle of pyrite, a common sulfide mineral.

$$2\text{FeS}_2\text{pyrite} + 2\text{H}_2\text{O} + 7\text{O}_2 \rightarrow 2\text{Fe}^{2+} + 4\text{SO}_4^{2-} + 4\text{H}^+$$

Equation 1: Pyrite Oxidation

$$4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$$

Equation 2: Ferrous to Ferric Iron

$$\text{FeS}_2\text{pyrite} + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+$$

Equation 3: Ferric Iron as Electron Acceptor

$$\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3\text{(aq)} + 3\text{H}^+$$

Equation 4: Hydrolysis Reaction

$$4\text{FeS}_2\text{pyrite} + 14\text{H}_2\text{O} + 15\text{O}_2 \rightarrow 4\text{Fe(OH)}_3\text{(aq)} + 8\text{SO}_4^{2-} + 16\text{H}^+$$

Equation 5: Overall Sequence

The acidic waters generated by the oxidation of sulfide minerals are generally referred to as acid mine drainage. The low pH of the water then allows for uranium and other heavy metals, including the daughter products of uranium decay, to solubilize. The dissolved uranium will generally occur in the form of $\text{UO}_2^{2+}$. During rain events or if the tailings are in communication with groundwater the acidic solution of uranium and other heavy metals is transported into surface and ground waters. As the solution moves away from the tailings pile the pH of
the solution will generally increase due to buffering by the carbonate system and weathering of silicate and oxide minerals. As the pH increases some of the dissolved uranium and heavy metals will form oxyhydroxides, and precipitate out of solution forming metal rich sediments. Essentially, the remaining portion of the dissolved uranium and heavy metals will complex with anionic ligands such as carbonate, phosphate and organic ligands to form ligand-metal complexes that stay in solution. These complexes can then travel through the ground and surface waters. The result is contamination from heavy metal laden sediments and complexed metals in the water.

Solution mining is done by injecting an oxidizing solution, generally containing oxygen and sodium carbonate into the subsurface, using wells that are drilled into uranium bearing ores (NIC 2003; US NRC 2005). The oxidizing solution leaches uranium in the form of uranyl tricarbonate ($\text{UO}_2(\text{CO}_3)_3^{4-}$) from the ore and the leachate is then extracted from the subsurface through wells (NIC 2003; US NRC 2005). This method can prove to be hazardous to the environment because the uranium is forced into solution, which poses the threat of escape from the site. If the recovery wells do not extract all of the dissolved uranium, the leachate may infiltrate to the groundwater and migrate with the groundwater forming a plume of contamination.

1.3.2 Conversion and Enrichment

During the processing stages of uranium conversion and enrichment, the possibility for contamination is also present. Releases of uranium during the processing are evident, considering that there are approximately 8 processing
facilities currently listed on the NPL (National Priorities List) (US EPA 2005). Several of the facilities are well known because of the extent of contamination present, these sites include US DOE sites at Hanford, Washington, Savannah River, South Carolina and Oak Ridge, Tennessee.

The first step in processing uranium is the conversion of the $\text{U}_2\text{O}_8$ “yellow cake” produced during the milling operation to uranium hexafluoride, $\text{UF}_6$ (UIC 2003b; US DOE 2005). The uranium hexafluoride is heated which causes it to become a gas and it is ready for enrichment (UIC 2003b; US DOE 2005). Enrichment with respect to $^{235}\text{U}$ is performed using either gaseous diffusion or gas centrifuge processes. The amount of $^{235}\text{U}$ enrichment is dependant upon the final use of the uranium. Most reactors need $^{235}\text{U}$ to be enriched from about 0.72% in natural uranium to about 3-5% depending on the reactor type. For uses as fission bombs $^{235}\text{U}$ must be enriched to 90% or greater in order to obtain the necessary critical mass. During the processing stages of uranium there is the possibility of releasing both liquid and gaseous $\text{UF}_6$. After the process is complete the uranium depleted with respect to $^{235}\text{U}$ is contained for disposal or shipped to plants for other uses in both military and civil applications.

1.3.3 Radioactive Waste Disposal

There are three types of radioactive wastes classified by the U.S. Nuclear Regulatory Commission, low-level waste (LLW), high-level waste (HLW), and uranium mill tailings (US NRC 2005). LLW generally includes items that have been contaminated through exposure to neutron radiation, medical waste and industrial waste (US NRC 2005). HLW generally consists of spent reactor fuel,
waste materials from spent fuel reprocessing, and materials from decommissioned nuclear weapons (US NRC 2005). Uranium mill tailings consist primarily of ore residues that contain radioactive decay products and heavy metals (US NRC 2005).

Disposal of LLW is generally by temporary storage on-site until the radioactive material has decayed sufficiently for disposal of the waste as municipal trash or the waste is disposed at a LLW disposal site (US NRC 2005). There are currently three LLW disposal facilities in the United States: the DOE facilities in Barnwell, South Carolina and Hanford Washington, and at Envirocare in Clive, Utah (US NRC 2005). There is not readily available documentation to determine whether there is substantial contamination caused by LLW storage/disposal facilities. LLW is commonly contaminated radioactive materials that decay quickly and if properly disposed of in a modern landfill the chances of groundwater and soil contamination is minimal.

Currently HLW is stored in spent fuel pools and dry cask storage at reactor sites around the country awaiting better treatment processes, availability of treatment facilities, or long-term storage (US DOE 1997; US NRC 2005). HLW is also stored at reprocessing facilities at DOE plants at West Valley, New York; Savannah River, South Carolina; and Hanford, Washington sites (US DOE 1997). The final long-term storage for much of the HLW waste is intended to be at the much-debated US DOE site in Yucca Mountain, Nevada (US DOE 2005; Wronkiewicz and Buck 1999).
There have been documented releases of HLW at DOE sites at Savannah River, South Carolina and Hanford, Washington (US EPA 2005). Both DOE sites that have had spills are on the NPL due to the severe extent of contamination present at the sites (US EPA 2005). There does not seem to be adequate documentation of releases of HLW pertaining to spent fuel pools and dry cask storage facilities at reactor sites to gage whether or not there is a problem or threat of contamination at these sites.

1.3.4 Military and Civil Applications of DU

There is a wide range of military and civil applications of uranium depleted with respect to the $^{235}$U isotope, commonly referred to as depleted uranium (DU). Depleted uranium is any uranium that contains less than the 0.72% $^{235}$U that is found in natural uranium. The use of DU is well known as a colorant in many products from glassware and ceramics to dentures (Betti 2003). In the past, depleted uranium was also widely utilized for dental procedures such as dental porcelains and crowns (Betti 2003). The use of DU in the dental industry ceased approximately 20-25 years ago (Betti 2003). Depleted uranium finds continued use as a chemical catalyst for large-scale industrial applications in the oil and gas industries (Betti 2003).

In the past DU was also used as radiation shielding for X-ray emitting devices and is currently used as radiation shielding in shipping containers (US NRC 2001; Bleise et al. 2003). There are approximately 15 shipping containers with several different designs that use DU alloys for gamma-ray shielding in order
to transport, store, and dispose of high-level radioactive wastes and/or spent nuclear fuel (US NRC 2001). Containers used to ship \(^{192}\)Ir are also lined with DU (US NRC 2001). Oak Ridge National Laboratory (ORNL) owns 11 such containers used to ship \(^{192}\)Ir to customers for use in radiography devices. Each of the containers owned by ORNL contains approximately 60 kg of DU.

The most widespread uses of depleted uranium take advantage of the density of metallic uranium (18.95 g/cm\(^3\)), which is approximately 60% more dense than lead (11.35 g/cm\(^3\)). The use of DU for high-density applications for civilian applications is primarily as counterweights and for military applications DU is used for high-density kinetic energy penetrators (Betti 2003; Bleise et al. 2003; Giannardi and Dominici 2003).

DU has been commonly used as counterweights for large bodied aircraft, forklifts and sailing yachts but is being replaced by tungsten (Betti 2003; Bleise et al. 2003). The amount of DU currently being used as counterweights is unknown but is decreasing; Table 3 indicates the use of DU counterweights in domestic US aircraft (US NRC 2001). The total tonnage of DU currently being used for counterweights in large bodied domestic US aircraft is approximately 379.65 metric tons.

<table>
<thead>
<tr>
<th>Aircraft</th>
<th>Number of Aircraft</th>
<th>Total Weight of DU Per Aircraft (kg)</th>
<th>Total Weight of DU (metric tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>McDonnell-Douglas DC-10</td>
<td>168</td>
<td>(\approx 1000)</td>
<td>(\approx 168)</td>
</tr>
<tr>
<td>Lockheed L-1011</td>
<td>60</td>
<td>(\approx 680)</td>
<td>(\approx 40.8)</td>
</tr>
<tr>
<td>Boeing B-747</td>
<td>201</td>
<td>(\approx 850)</td>
<td>(\approx 170.85)</td>
</tr>
</tbody>
</table>

Table 3: Tonnage of DU used as counterweights in US domestic aircraft (US NRC 2001)
The use of DU as kinetic energy penetrators began with the US Army in the early 1970s and continues today (Bleise et al. 2003). DU penetrators are most commonly used as munitions for the 30 mm GAU-8 cannon on the Air Force A-10 Thunderbolt II (warthog), the 25 mm cannon on the US Marine Corp AV8-B Harrier, and for the 120 mm sabot round used in the M1A1 Abrams Tank (US DOD 2005). During the Bosnia-Herzegovina conflict in 1994 approximately 10,000 30 mm DU rounds were fired at 12 locations, which amounts to about 3.3 tons of DU munitions (US DOD 2005). In 1999, during the Kosovo conflict approximately 31,000 30 mm DU rounds were fired at 85 locations amounting to about 10.2 tons of DU munitions (US DOD 2005). The largest use of DU munitions in wartime or peacekeeping operations by the US military was during the Gulf War in 1990-1991, except perhaps during the second Gulf War, which continues today. During the first Gulf War approximately 260 tons of 30 mm rounds, 50 tons of 120 mm sabot rounds, and 10 tons of 25 mm rounds were fired during the conflict.

There are a variety of industrial and medical uses for DU, which poses a threat to the potential contamination of water resources and exposure from accidental and/or intentional releases and improper disposal. Improper disposal of DU can apply to disposal of DU containing products from ballast weights to glassware and ceramics. Another concern is the accidental release of DU from ballast weights in aircraft arising from crashes (Betti 2003). A more imminent concern of DU dispersion comes from the use of DU in munitions used by NATO and US forces around the globe, but more importantly on the battlefield in
conflicts in Iraq (two separate conflicts), Kososvo, and Bosnia. The disposal and dispersal of DU poses a threat to human health and the environment from human exposure and from the possibility of DU dissolution and infiltration into groundwater and surface water (Betti 2003; Durante and Pugliese 2003; Giannardi and Dominici 2003).

1.4 Remediation Methods

1.4.1 Ex-Situ Methods

The primary conventional remediation technology used for treating U-contaminated groundwater is the pump and treat method. This process involves removal of the contaminated groundwater from the subsurface and then separation and disposal of the contaminants at the surface. Some common techniques for separating U from the water include: bioremediation (bioreductive precipitation, adsorption, and bioaccumulation), chemical treatment, ion exchange, and reverse osmosis.

Bioreductive precipitation is discussed further in subsequent sections of this thesis (1.5 Dissimilatory Metal Reduction and 1.6 U(VI) Reductive Precipitation). Biosorption is a process by which contaminants, specifically metals that include U, can be adsorbed onto the wall surfaces of microorganism cells (Abdelouas et al. 1999a). The primary purpose of biosorption technologies that have been developed is to remove metals during water reclamation processes in ex-situ applications (Abdelouas et al. 1999b). Bioaccumulation generally refers to a process where metals are precipitated from solution and
accumulate upon cells. A pertinent example of bioaccumulation is the precipitation of $\text{HUO}_2\text{PO}_4$ on the surface of *Citrobacter* sp. cells (Macaskie et al. 1992; Finlay et al. 1999).

Chemical treatment of U contaminated water generally employs chemically promoted reductive precipitation by the addition of certain catalysts that cause U(VI) to become reduced to U(IV). Once the uranium precipitates into the reduced form it settles out of solution in a settling tank used as part of the water reclamation process. Some common chemical catalysts for removing U(VI) from contaminated water include ferrous and zero-valent iron (Lee and Bondietti 1983; Abdelouas et al. 1999b; Liger et al. 1999). Chemical coagulation of U(VI) is also a method which may use ferric iron and alum as coagulants. Reduction by zero-valent iron and alum coagulation has been shown to be quite effective by removing up to 95% of available uranium from solution (Lee and Bondietti 1983; Abdelouas et al. 1999b). Ferrous iron is somewhat less effective, but still removes up to 93% of available uranium from solution (Lee and Bondietti 1983). Ferric iron coagulation has been shown to be the least effective of the chemical treatment techniques, removing up to 80% of available uranium from solution (Lee and Bondietti 1983). Many of the pilot studies used to determine the effectiveness of *ex-situ* uranium removal from contaminated water were performed in the early 1980’s at Oak Ridge National Laboratory and New Mexico State University (Lee et al. 1982; Lee and Bondietti 1983; White and Bondietti 1983; Hanson et al. 1987).
Ion exchange is a process that has been around for many years and has applications ranging from domestic uses as water softeners to industrial uses for recovering uranium from water at uranium mines (Ross and George 1971). There have been many studies and pilot plant tests in order to determine whether the optimum removal of uranium occurs using anion exchange resin or cation exchange resin. It has been demonstrated that H\(^+\) cation exchange resins are the most efficient with 93-97% uranium removal rates. Other types of cation exchange resins and anion exchange resins have lower removal rates (Ross and George 1971; Lee et al. 1982; Lee and Bondietti 1983; Hanson et al. 1987; Varani et al. 1987; Jelinek and Sorg 1988; Sorg 1988).

It has been suggested that the efficiency of the H\(^+\) cation exchange resin is made possible because the uranium is commonly present as uranyl carbonates (Lee and Bondietti 1983; Lee et al. 1982). The uranyl ion is likely substituted with H\(^+\) allowing bicarbonate to proceed through the resin bed while the uranyl ions remain attached to the resin (Lee et al. 1982; Lee and Bondietti 1983). Once resins become saturated with the uranyl ion the resins must be regenerated by removing the uranyl from the resin, which is performed by flushing with either 10% NaCl or with 4% NaOH and 1N HCl (Sorg 1990). The other common technique for removing uranium from waste streams and in water reclamation processes is via reverse osmosis.

Reverse osmosis (RO) is a process where contaminated water is pressurized and passed through a semi-permeable membrane, which separates the contaminants from the water (Abdelouas et al. 1999a). RO systems are
widely utilized as water filtration devices in households as well as for desalination for domestic water supplies worldwide (Pantell 1993). Reverse osmosis desalination systems are utilized in many areas around the world where fresh water is not available (Pantell 1993). In the past poor efficiency and high operation costs of reverse osmosis systems has prevented them from being widely utilized, but as RO systems become more efficient and less costly to operate they are being employed more frequently in the U.S. and abroad (Pantell 1993). Laboratory studies have indicated that household RO systems are quite efficient at uranium removal with greater than 99% removal rates (Fox and Sorg 1987). Pilot plant studies have shown that uranium can be removed from groundwater with great success using reverse osmosis filtration systems with a 99% removal rate (Huxstep and Sorg 1987). Beyond the conventional treatment methods of bioremediation, chemical treatment, ion exchange, and reverse osmosis there are emerging in-situ technologies that are advancing uranium remediation efforts.

1.4.2 In-Situ Methods

Some of the more promising emerging technologies for in-situ uranium mitigation include: in situ vitrification (ISV), phytoremediation, in situ redox manipulation (ISRM)/bioremediation, and permeable reactive barriers (PRB). Each of these methods shows promise to be a cost effective option for uranium as well as other metal and radionuclide remediation. Each method has been tested to some extent but all still need to be perfected and the limitations and advantages need to be determined in order to make them more widely utilized.
In situ vitrification (ISV) is a process where electrodes are inserted into the subsurface and alternating current (AC) is applied to the electrodes causing the soil between the electrodes to become heated (National Research Council 1999). The soil between the electrodes can reach temperatures exceeding 1700°C causing the soil to melt. Upon cooling, the mass becomes an impermeable glass or crystalline monolith trapping metals (National Research Council 1999). ISV has had successful applications at sites around the globe including 20 pilot scale tests and 6 large-scale tests at the DOE Hanford Site in Hanford, WA where 9 radionuclides and 13 metals were vitrified (National Research Council 1999). ISV can also be used to mitigate sites containing organic compounds as well as metals and radionuclides (National Research Council 1999). There are several limitations to ISV which include: the depth must be less than 6 meters, it cannot be used if there is excessive moisture (i.e. groundwater) especially if volatile organic compounds are present, it cannot be used if underground utilities are nearby, and soil organic content should be less than 7-10 percent by weight (National Research Council 1999). Another type of in situ remediation method available to target near surface contaminated sediments, but over larger areas is phytoremediation.

Phytoremediation is a process that utilizes plants that hyperaccumulate heavy metals in order to cost effectively remove metals from soil (Cornish et al. 1995). The phytoremediation process occurs when plants release chelating agents from their roots in order to complex micronutrients in the soil, which also chelates metals allowing the plants to uptake both the micronutrients and the
metals (National Research Council 1999). In order for phytoremediation to be used it is best if the targeted contamination exists within shallow surface soils over a large area with low to moderate concentrations of metals (National Research Council 1999). Pilot studies using *Trifolium pratense* (red clover) and *Descurainia pinnata* (tansy mustard) have displayed promising results with up 10 mg U/kg of leaf and stalk biomass being collected (Abdelouas et al. 1999b). The most promising hyperaccumulators for use in phytoremediation are reported to be of the genera *Brassica*, *Thlaspi*, *Cardaminopsis*, and *Alyssum* (Kumar et al. 1995 and Ahmann 1997). Another type of *in situ* remediation method available to target deeper sediments is *in situ* redox manipulation (ISRM).

*In situ* redox manipulation (ISRM) and bioremediation are very closely related in regards to uranium remediation. ISRM is a method where chemical reductants are injected into the subsurface, assuming that the aquifer is an oxidizing environment, which is generally the case, causing the reduction and immobilization of redox sensitive contaminants (Amonette et al. 1994; Fruchter et al. 1996; Fruchter et al. 1997; Sorg 1999; Abdelouas et al. 1999a). Bioremediation, in the case of redox sensitive contaminants such as uranium, also relies on reductive precipitation in order to immobilize contaminants (see also: 1.6 Dissimilatory Metal Reduction and 1.5 Reductive Precipitation of U(VI)). It is possible to essentially couple the two methods by injecting lactate in order to stimulate native MRB (Metal Reducing Bacteria), but no such definitive field studies exist in regards to redox sensitive contaminants (Fruchter et al. 1997). Field studies using ISRM were performed at the DOE Hanford site by injecting
Na dithionite (Na$_2$S$_2$O$_4$) into the subsurface targeting hexavalent chromium (Fruchter et al. 1996; Fruchter et al. 1997; and Scott et al. 1998). Results of the tests indicate that approximately 60-100% of the Fe in clays was reduced based upon core data and there did not appear to be any significant plugging of pore space within the aquifer formation (Fruchter et al. 1996; Fruchter et al. 1997; Scott et al. 1998). As a result of the ISRM treatment using Na dithionite the hexavalent Cr concentration at the study site went from an initial concentration of 60 $\mu$g/L to below detection limits after treatment (Fruchter et al. 1996; Fruchter et al. 1997; Scott et al. 1998). ISRM is somewhat similar in function to a permeable reactive barrier in that an area of the subsurface is turned into a permeable treatment zone, which ideally transects a contaminant plume.

A permeable reactive barrier (PRB) is a trench backfilled with reactive material that transects perpendicular to the flow of groundwater intersecting a contaminant plume (Abdelouas et al. 1999a; Sorg 1999). There are many different reactive materials that may be added to the trench in order to effectively remove the contaminant from the groundwater plume. Abdelouas et al. (1999a; b) suggests that zero valent iron (Fe$^0$) is a preferred material for backfilling the trench or as an injection of Fe$^0$ colloids because it effectively immobilizes uranium via reductive precipitation, as well as Mo, Tc, Cr and is also able to degrade chlorinated hydrocarbons. A PRB field study to remove uranium from groundwater in Fry Canyon, Utah tested zero valent iron and found that more than 99.9% of the uranium was removed from the groundwater passing through the PRB (Naftz et al. 2000). Permeable reactive barriers with bone char
phosphate and amorphous ferric oxyhydroxide (AFO) as the reactive materials were also tested at the Fry Canyon site and were found to remove greater than 70% of the incoming uranium from the groundwater (Naftz et al. 2000). Morrison and Spangler (1992) surveyed 24 different materials for use in permeable reactive barriers to treat uranium contamination from uranium mill tailings. The results from Morrison and Spangler (1992) indicate that hydrated lime (Ca(OH)$_2$), fly ash, barium chloride (BaCl$_2$$\cdot$$2$H$_2$O), calcium phosphate (Ca$_5$(PO$_4$)$_3$OH), titanium oxide (TiO$_2$), peat, and lignite were able to remove greater than 99 percent of the dissolved uranium from solution via sorption or U(VI) complexation. In PRB systems where the reactant sorbs uranium such as peat, hematite, ferric oxyhydroxide, titanium oxide and designer sorbates such as polymer-coated silica, uranium removal may be efficient but the sorbed uranium is sensitive to chemical fluctuations in the passing groundwater and may be desorbed (Morrison and Spangler 1992; Bryant et al. 2003; Fuller et al. 2003;). The preferred reactants would be ones that do not simply sorb the uranium but remove it from solution as a precipitate, as is the case with zero valent iron, hydrated lime, fly ash, and calcium phosphate (hydroxyapatite) (Morrison and Spangler 1992; Arey et al. 1999). Zero valent iron causes U(VI) to precipitate as a poorly crystallized hydrated uraninite (UO$_2$$\cdot$$n$H$_2$O) (Abdelouas et al. 1999b). The addition of hydrated lime to a solution containing U(VI) results in the precipitation of an X-ray amorphous precipitate, which is likely a calcium uranate (ex. CaUO$_4$). The addition of fly ash results in a similar precipitate, which is also likely a calcium uranate (Morrison and Spangler 1992). The addition of calcium
phosphate into solution containing U(VI) results in precipitation of what is likely secondary phosphate phases (Arey et al. 1999). The hydrated lime, fly ash, and calcium phosphate additions all result in U(VI) precipitates whereas the addition of zero valent iron results in U(VI) reduction causing a U(IV) precipitate.

1.5 Dissimilatory Metal Reduction

Dissimilatory metal reduction is a process by which specialized microorganisms are able to respire metals. These microorganisms are able to use a wide range of metals and metalloids as terminal electron acceptors. Some of these terminal electron acceptors include: Fe(III), Mn(IV), U(VI), Se(VI), Se(IV), Se(0), Cr(VI), Hg(II), Tc(VII), V(V), Mo(VI), Cu(II), Au(III), Au(I), and Ag(I) (Lovley 1993b). The process by which the microorganisms access the metal is somewhat unclear but what is known is that the metal acts as the terminal electron acceptor in the process and some other substance must act as an electron donor (Nealson et al. 2002; Luu and Ramsay 2003).

It is suggested that electron shuttling may play an important role in dissimilatory metal reduction (Lovley et al. 1996; Luu and Ramsay 2003). When the oxidized state of a metal is quite soluble, which is the case for uranium, the way in which the metal reducing bacteria (MRB) accesses the metal is not as significant as in situations that involve an electron acceptor with a very low solubility, such as Fe(III) and Mn(IV). At neutral pH, Fe(III) and Mn(IV) are generally present as highly insoluble oxides and the mechanisms by which MRB access these oxides is unclear. Nealson et al. (2002) suggests that the pathways
for reduction of these oxides may be different for each metal or even for different oxides of the same metal. Luu and Ramsay (2003) similarly suggest that different pathways for dissimilatory metal reduction exist for different bacteria.

The Luu and Ramsay (2003) predictions are made for two Fe(III)-reducing bacteria, *Shewanella* and *Geobacter* species. It is indicated that *Geobacter* species must have direct cell-oxide contact for reduction to occur unless an exogenous electron shuttle is present, such as anthraquinone-2,6-disulfonate (AQDS) (Luu and Ramsay 2003). Unlike *Geobacter* species, *Shewanella* species have the capability to produce extracellular electron-shuttling compounds, which allows *Shewanella* species at least two pathways for Fe(III) reduction (Luu and Ramsay 2003). The other pathway for *Shewanella* species beyond the extracellular shuttling compounds is direct contact between the oxides and the membrane-bound cytochromes (Luu and Ramsay 2003). The manner in which bacteria access metal-oxides is a phenomenon about which little is known. The exact process of metal-oxide reduction is unclear but new information will likely be presented in the future as more studies investigate new mechanisms for this phenomenon and more clearly assess the currently accepted mechanisms. Most of the current research has focused on the reduction mechanisms associated with Fe(III) oxide reduction and much less is known about the mechanisms involved with other metal reduction (Urrutia et al. 1998; Das and Caccavo 2000; Turick et al. 2002).
1.6 Microbial U(VI) Reductive Precipitation

Previous studies indicate that U(VI) reduction can occur by metal reducing bacteria (MRB) such as *Shewanella putrefaciens* and *Geobacter metallireducens* (Lovley and Phillips 1992). The aforementioned microorganisms have the ability to use U(VI) as a terminal electron acceptor (TEA) coupled with organic carbon or H$_2$ as an electron donor in order to obtain energy for growth (Lovley et al. 1991). Sulfate-reducing bacteria (SRB), such as *Desulfovibrio desulfuricans*, also have the ability to reduce U(VI) (Lovley and Phillips 1992). Many SRB can reduce U(VI) but lack the ability to utilize U(VI) as the sole TEA in order to harness energy for growth. The SRB *Desulfitomaculum reducens* strain MI-1 has been demonstrated to have the ability to utilize U(VI) as the sole electron acceptor in order to grow (Tebo et al. 1998). Other bacterial isolates that can reduce U(VI) include Clostridium *sp.* (Francis et al. 1994) and *Deinococcus radiodurans* R1 (Fredrickson et al. 2000). These bacteria, like many known SRB, cannot use U(VI) as the sole TEA.

Previous work by Ganesh et al. (1997) and Robinson et al. (1998) indicates that the microbial reduction rate of U(VI) from organic complexes is dependant upon the organic ligand present. Haas and DiChristina (2002) obtained similar results using the facultative MRB *S. putrefaciens* to reduce Fe(III) complexed with a wide range of organic ligands. Haas and DiChristina (2002) reported that Fe(III) reduction is controlled by competitive speciation among dissolved organic ligands and functional groups on the cell surface. It was observed that the enzymatic Fe(III) reduction rates correlate with the
equilibrium stability constants for Fe(III)-organic ligand complexes (Haas and DiChristina 2002). It is not known whether the same type of correlation is true for U(VI)-organic ligand complexes. In order to assess uranium contamination and remediation it is important to first understand the bioavailability of U(VI) in uranyl-organic complexes.

The purpose of this study was to understand how U(VI) complexation affects U(IV) reductive precipitation with respect to *S. putrefaciens*. A variety of organic ligands that differ significantly in structure and with respect to the stability of the U(VI)-organic complex were selected. The ligands used in this study include; glutaric, adipic, pimelic, succinic, maleic, malonic, oxalic, citric, nitrotriacetic (NTA), 4,5-dihydroxy-1,2-benzendisulfonic (Tiron), Aldrich brand humic, and ethylenediaminetetraacetic (EDTA) acids. The experimental microbial U(VI) reduction rates were obtained throughout the course of this experiment and compared with the equilibrium stability constants of the U(VI)-organic ligand complexes in order to better understand the bioavailability of U(VI) in natural settings.
2. METHODS

2.1 Bacteria Selection and Media Composition

For this study *Shewanella putrefaciens* strain 200R was employed to use U(VI) as the TEA and ultimately reduce hexavalent uranium to tetravalent uranium. *S. putrefaciens* is a gram-negative metal-reducing facultative anaerobe. This bacterium was chosen because it is a fairly robust MRB capable of using hexavalent uranium as a TEA and is commonly found in natural subsurface settings.

Stock cultures of 200R were maintained by freezing in 15% glycerol at -80°C. The freezer stock were used to inoculate Luria-Bertani (LB) agar plates, which were grown at 30°C aerobically for about 24 hours until colonies formed. The 200R grown for experiments were cultured by inoculating 500 ml of sterilized liquid LB media with a single colony from an active LB agar plate. The experimental cultures were grown aerobically for about 24 hours at 30°C on a shaker. The active culture was harvested at mid log phase by centrifugation and washed two times using sterile 0.1 M NaCl and then the cells were resuspended in sterile 0.1 M NaCl for use as inoculum.

The experimental media was a modified form of the *Geobacter* freshwater media from Lovley et al. (1991). The composition of the media was: NH₄Cl 0.25 g/L, KCl 0.1 g/L, Na-lactate (60% syrup) 5 ml/L, Sigma ® RPMI-1640 vitamin solution 0.1 ml/L, and modified Wolfes mineral solution 1 ml/L. Refer to Appendix A for the composition of the RPMI-1640 vitamin solution and the modified Wolfes mineral solution. Uranyl acetate and selected chelating ligands
were added in the following proportions: 1 mM uranyl acetate and 200 mM chelating ligand, 100 μM uranyl acetate and 20 mM chelating ligand and 10 μM uranyl acetate and 20 mM chelating ligand. The pH of the experimental media was adjusted to 7 using sterile trace metal grade NaOH and HCl.

2.2 Ligand Selection

The ligands used in this study were selected on the basis of developing a data set of common organic acids with a wide range of complexation stability constants in relation to the uranyl-ligand complex. The humic acid was used in this study in order to determine what bioavailability differences exist between native organic matter (NOM) complexes and simple organic ligand complexes.

The JCHESS algorithm was used in order to determine the concentrations of organic ligand to use with each U(VI) concentration used in this study (van der Lee and De Windt 1999). Specifically, the speciation calculations performed using JCHESS used the concentration of all organic and inorganic species present in the media. These speciation calculations were completed so that the ligand concentration for each of the three uranyl concentrations could be tailored to ensure that >90% of the U(VI) in each solution was complexed by the intended ligand. Refer to Appendices B-D for the results of the JCHESS speciation calculations.

2.3 Experimental Setup

The experimental media used in U(VI) reduction experiments was sterilized and transferred into a Coy® anaerobic chamber in 50mL centrifuge
tubes, which were each bubbled with the internal air (N₂ 85%, CO₂ 10% and H₂ 5%) for 2-3 minutes. These experiments were set up as a series of batch reactors with a different ligand-uranyl complex in each 50 mL centrifuge tube. The experimental media containing each U(VI)-ligand combination was placed on a low speed shaker and allowed to equilibrate for 24 hours before the batch reactors were inoculated.

After the 200R culture was washed twice in sterile anoxic 0.1 M NaCl and resuspended in sterile anoxic 0.1 M NaCl, it was transferred to the anaerobic chamber and flushed with the internal air for 3-5 minutes in order to remove any dissolved oxygen from the bacteria culture. Immediately after the active culture was washed and degassed each batch reactor containing the uranyl-ligand complexes was inoculated with 1 mL of bacteria culture per 50 mL of media. The experiment was carried out in the anaerobic chamber, maintained at 30°C, with the tubes gently shaking.

2.4 Sampling and Analysis

At timed intervals throughout the experiment each batch reactor was sampled by drawing 1 mL of sample out of each 50 mL centrifuge tube using a syringe. The 1 mL sample was then filtered using a 0.2 µm syringe filter. The sample was then acidified using trace metal grade nitric acid. After acidification the samples were removed from the anaerobic chamber, diluted and spiked with Dy as an internal standard and analyzed by ICP-MS. Analysis by ICP-MS provides concentration of total U remaining in solution.
3. RESULTS

The results of U(VI)-ligand complex reduction experiments are displayed in Figures 1, 2 and 3. Each figure displays a different combination of U(VI) and ligand concentrations: 10 µM U(VI) and 20 mM ligand (Fig 1), 100 µM U(VI) and 20 mM ligand (Fig. 2) and 1 mM U(VI) and 200 mM ligand (fig. 3).

![Graph showing the percentage of U remaining in solution over incubation time for different ligands](image)

**Figure 1:** 10 µM U(VI) and 20 mM ligand
Figure 2: 100 μM U(VI) and 20 mM ligand

Figure 3: 1 mM U(VI) and 200 mM ligand
3.1 Results for 10 µM U(VI) and 20 mM Ligand

Figure 1 displays data for the U(VI) reduction experiments in which the initial U(VI) concentration was 10 µM and 20 mM of ligand was added. Error is approximately 5% for all trials except for humic acid. Error bars for the humic acid is an estimate of uncertainty based upon replicate analyses. The figure displays the percentage of U(VI) remaining in solution as a function of time. In this set of experiments reduction was rapid in the inoculated control tube, which contained lactate as a carbon source and no other organic ligands. This was consistent with the formation of a black U(IV) precipitate, likely an amorphous UO$_2$ precipitate (UO$_2$(am)), on filter membranes during sampling. During all subsequent experiments the same black precipitate was a visual indicator of uranium reduction, which was then confirmed by ICP-MS analyses for U in solution. The U(IV) precipitate could be seen not only on filters, but also directly in the batch reactor vessels of experiments done at 100 µM and 1 mM U(VI) concentrations. Initial U(VI) reduction rates for the 10 µM U(VI) inoculated control sample is very rapid with reduction of >80% of the available U(VI) occurring within the first hour of incubation. Within 24 hours of incubation time ~ 95% of the available U(VI) from solution was reductively precipitated. From this 24 hour mark through the end of the experimental sampling period (96 hours) little if any additional reduction occurred. During the initial reduction period, the first 2-3 hours of incubation, U reduction in the control is essentially linear with time, and then reduction rates begin to decrease asymptotically.
The batch experiments with pimelic, adipic, glutaric and maleic acid complexed U displayed similar behavior as the inoculated control. Each had significant initial reduction rates where about 80% of the available U(VI) was reductively precipitated. After that time the reduction rates began to decrease asymptotically, as with the inoculated control. Like the inoculated control, after 24 hours of incubation reduction did not proceed further and ~10% U remained in solution for all of these ligands.

The experiments containing malonic and humic acids displayed lower rates for reductive precipitation of U. The U in solution in the malonic acid sample slowly decreased down to about 20% U remaining in solution by the end of the experiment at 96 hours. The U in the humic acid batch reactor was reduced to about 50% of the initial concentration within the first 24 hours of incubation and remained at that level throughout the remainder of the experiment.

The oxalic acid, citric acid, tiron, NTA and EDTA batch reactors did not show any clear evidence of reductive precipitation throughout the 96 hour sampling period.

3.2 Results for 100 $\mu$M U(VI) and 20 mM Ligand

The data for the 100 $\mu$M U(VI) and 20 mM ligand reduction experiments displayed in figure 2 shows similar reduction trends as the 10 $\mu$M U experiments. Error is approximately 5% for all trials except for humic acid. Error bars for the humic acid are estimated based upon replicate analyses. The inoculated control, and experiments with pimelic and adipic acids have very similar reduction rates,
with removal of nearly all U from solution by the 96 hour end mark. The glutaric, succinic and malonic acid batch experiments had a slightly slower initial reduction period but still led to removal of nearly all U from solution by the end of the sampling period. In the first 10 hours of the experiment, U complexed with maleic acid was reduced until ~60% of the initial U remained in solution, at which point further reduction was slow or nonexistent. Addition of oxalic, citric and humic acids as well as tiron, NTA and EDTA completely arrested U reduction over the course of the experiments (96 hours).

3.3 Results for 1 mM U(VI) and 200 mM Ligand

The 1mM U(VI) and 200 mM ligand data presented in figure 3 display trends that are somewhat similar to those in the previous two figures. Error is approximately 5% for all trials. However, the initial reduction rate for the control was not as fast as in the previous sets of experiments. The U(VI) reduction in the control, and in experiments with pimelic, adipic and glutaric acids all proceeded until U remaining in solution was below detection limit at the end of the 96 hour sampling period. The batch reactors containing succinic, malonic, maleic and oxalic acids displayed reduction in the first 10 hours, removing ~20% of the initial U in solution. After 96 hours, the percentage of the U(VI) remaining in solution was 20%, 50%, 70% and 80% for each of these ligands, respectively. The citric acid, tiron, NTA and EDTA samples did not display any appreciable U reduction. Humic acid data was not obtained for the 200 mM ligand concentration due to problems with filtering the samples.
4. DISCUSSION

4.1 Reductive Precipitation Rates

Figures 4 and 5 show the U(VI) reduction rate as a function of the stability of the 1:1 aqueous U(VI)-organic complex. Figure 4 displays the U(VI) reduction rate as an actual rate (mM/hr), whereas in Figure 5 the U(VI) reduction rate is displayed as a relative rate (% per hr). Each figure contains data from all three experimental concentrations: 10 µM U(VI) and 20 mM organic ligand, 100 µM U(VI) and 20 mM organic ligand and 1 mM U(VI) and 200 mM organic ligand. Figure 5 also contains the values for humic acid, shown as black symbols. The data for Figures 4 and 5 are initial reduction rates taken from the first 8-9 hours of each experiment when the rate of reductive precipitation of U(IV) was approximated as being linear. The initial rates were utilized in order to avoid the effects of Michaelis-Menten kinetics at later points (>9 hrs.) in the experiments. Further discussion regarding the effects of Michaelis-Menten kinetics in this study will be presented in 4.2.

The best fit regions of linear reduction rates displayed in Figure 4 as a function of the 1:1 U(VI)-ligand stability constant are analogous to those observed previously for Fe(III) reduction (Haas and DiChristina 2002). The trends in Figure 4 also indicate that U reduction rate is dependent upon the initial concentration of U(VI) in solution, which is consistent with Michaelis-Menten kinetics where the lower U(VI) concentration (terminal electron acceptor) would be the limiting factor in terms of overall reduction rate.
Figure 5 displays the relative U(VI) reduction rates as a function of the 1:1 U(VI)-ligand stability constant. The similar trend observed for all three metal:ligand ratios demonstrates that complexation has a strong influence on uranium reduction rates, and furthermore, that the 1:1 U(VI)-ligand stability constant can provide a quantitative estimate for the potential relative rate of reduction for a given system.

All of the experimental results described in Chapter 3.0 suggest that U(VI) reduction by *S. putrefaciens* is strongly dependant on U(VI) solution speciation. Previous work by Haas and DiChristina (2002) indicates that Fe(III) reduction rates by *Shewanella putrefaciens* also varies as a function of the 1:1 Fe(III)-organic ligand stability constant. It was demonstrated that this relationship is comprised of three primary regions (see Figure 2 in Hass and DiChristina 2002): a region with low Fe(III)-ligand stability constants and high Fe(III) reduction rates, a region with high Fe(III)-ligand stability constants and arrested Fe(III) reduction rates, and finally a transitional region connecting the others, where an edge of rapidly increasing Fe(III)-ligand stability constants results in rapidly decreasing Fe(III) reduction rates. The data in the present study displays a similar relationship, which is illustrated in Figure 5. Based on the similarities between the present study and the study by Haas and DiChristina (2002) it seems that U(VI) reduction rates, like Fe(III) reduction rates, vary as a function of the 1:1 metal-ligand stability constant.
Figure 4: Reduction rates for 1:1 U(VI)-ligand complex

Figure 5: Relative reduction rates for 1:1 U(VI)-ligand complex. Black symbols are humic acid values.
4.2 Michaelis-Menten Kinetics

The rate of enzyme catalyzed reactions is often described by Michaelis-Menten kinetics. In a system where Michaelis-Menten mechanics control the reaction, the rate of reaction is dependent upon the concentration of substrate, $S$, which is present in excess with respect to an enzyme or other catalyst, $E$. The system will follow the Michaelis-Menten equation (see Equation 6), where $v = $ reaction rate, $[S] = $ substrate concentration, $V = $ maximum rate, and $K_m = $ the Michaelis-Menten constant where substrate concentration is at half the maximal velocity ($V$).

$$v = \frac{V[S]}{K_m + [S]}$$

Equation 6: Michaelis-Menten Equation

This equation is also applicable in situations where an enzyme or catalyst, $E$, is present is excess over the substrate, $S$, in which case the concentration of the enzyme or catalyst, $[E]$, will appear in the equation rather than the concentration of the substrate, $[S]$. The $V$ and $K_m$ parameters can be evaluated using the slope and intercept of a linear plot, either a Lineweaver-Burk plot ($v^{-1}$ vs. $[S]^{-1}$) or an Eadie-Hofstee plot ($v$ vs. $v/[S]$) (Millar 2000).

The asymptotical decrease in reduction visible in Figures 1-3 indicates that Michaelis-Menten kinetics were a factor in this study. The decrease in reduction was likely the result of decreasing electron donor and acceptor (lactate and U(VI) respectively) concentrations causing these compounds to become limiting factors of U(VI) reduction. In order to avoid the results of Michaelis-Menten kinetics only the initial, linear, rates were used in Figures 4 and 5. The
correlation of initial U reduction rate to initial U(VI) concentration for the inoculated control samples is displayed in Figure 6. The linear trend line indicates that the maximum reduction rates were not reached in this study with the maximum U(VI) concentration of 1 mM. The maximum reduction rates would be marked by an asymptotical increase of U reduction rates causing a plateau feature on the figure depicting U reduction rate as a function of initial U(VI) concentration. The U reduction rates in the media amended with organic ligands were slower than the inoculated control, indicating that maximum reduction rates imposed by Michaelis-Menten kinetics was not a controlling factor in initial reduction rates, rather U speciation effects were the underlying cause.

![Figure 6: Initial reduction rates as a function of initial U(VI) concentration in inoculated control](image)

Reduction Rate = 0.0716[U(VI)] + 0.0009

$R^2 = 0.9995$
4.3 Alternate Hypothesis

The results of this study are likely, at least in part, a result of the 1:1 U(VI):ligand stability constant as was presented above (see 4.1). However, another contributing factor to the results seen in this study may be the chelation of U(IV) by the added organic ligands. It is difficult to assess the extent of U(IV) chelation effects on the rate of UO$_2$(am) precipitation due to the lack of reliable equilibrium constants in the literature for U(IV) aqueous complexation. This alternate hypothesis is supported by articles in the literature that had similar findings (Ganesh et al. 1997; Haas and Northup 2004; Robinson et al. 1998). Ganesh et al. (1997) found that solutions containing tiron had little U(IV) precipitate from solution in the presence of Desulfovibrio desulfuricans and Shewanella alga. Haas and Northup (2004) reported trends of retarded precipitation of UO$_2$(am) based on chelation for a variety of ligands using S. putrefaciens. Robinson et al. (1998) found that U(IV) readily complexed with citrate in the presence of D. desulfuricans, preventing the precipitation of UO$_2$(am). Based on the results found in the literature and the results of this study it is quite likely that U(VI) bioreductive precipitation is in part due to 1:1 U(IV)-ligand aqueous complexes.

4.4 Implications

The results of this study can be used to better understand the role of organic ligands with respect to bioavailability of U(VI) and other potential TEAs. It is clear that there is a link between the 1:1 U(VI):ligand stability constant and reduction rates but what is not evident is to what extent the chelation of U(IV) by
the ligands effects the reductive precipitation of UO$_2$(am). This role of U(IV) chelation could also have significant implications with respect to the natural behavior of U as well as to the use of bioremediation efforts in regards to U remediation. The lack of precipitation of bioreduced U in the presence of organic chelating agents may inhibit the formation of U ore bodies. In terms of remedial action the precipitation of UO$_2$(am) is necessary to stop the migration of highly soluble U(VI) and without the precipitation of U the chelated U(IV) would remain in solution and be able to continue to migrate. Haas and Northup (2004) estimated the 1:1 log K for U(VI):humic acid to be approximately 9, which is displayed in Figure 5. Based on Figure 5, humic acid follows a similar trend as other intermediate to strongly chelating organic acids. This observation would suggest that the presence of dissolved organic material could prevent U precipitation, undermining bioremediation efforts. This observation also explains results from Zielinski and Meier (1988) which indicate that hexavalent uranium can exist in peat bogs even when the conditions are reducing.

4.5 Future Work

Future research in order to better understand the results of this study would need to include the determination of U(IV) aqueous complexation data in order to more thoroughly assess the impact of U(IV) chelation with respect to the reductive precipitation of U. If the impacts of U(IV) chelation were more thoroughly documented it would allow for a more complete understanding of the bioavailability of U(VI) with respect to U(VI) speciation in the presence of organic...
chelating ligands. It would also be beneficial in the future to perform a similar study where U(VI) and U(IV) were measured during the experiment in order to better determine the overall speciation of U.
APPENDIX A

Composition of Mineral Solution and Sigma® RPMI-1640 Vitamin Solution

The tables presented in this appendix indicate the relative composition of the mineral and vitamin solutions used in the preparation of the modified Geobacter freshwater media used in the U(VI) reduction experiments. The mineral solution is a modified form of the Wolfes mineral solution as per Lovley et al. (1991). The vitamin solution comes prepared from Sigma® as the RPMI-1640 Vitamin Solution.
### Mineral Solution (10x)

<table>
<thead>
<tr>
<th>Components</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$EDTA $\cdot$ $2$H$_2$O</td>
<td>2.6000</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>0.3700</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0600</td>
</tr>
<tr>
<td>FeSO$_4$$\cdot$$7$H$_2$O</td>
<td>0.1700</td>
</tr>
<tr>
<td>CoSO$_4$(CoCl$_2$$\cdot$$6$H$_2$O)</td>
<td>0.1200</td>
</tr>
<tr>
<td>Ni(NH$_4$)$_2$(SO$_4$)$_2$$\cdot$$6$H$_2$O</td>
<td>0.2000</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$$\cdot$H$_2$O</td>
<td>0.1000</td>
</tr>
<tr>
<td>Na$_2$SeO$_4$(anhyd)</td>
<td>0.0280</td>
</tr>
<tr>
<td>MnSO$_4$$\cdot$H$_2$O</td>
<td>0.0220</td>
</tr>
<tr>
<td>ZnSO$_4$$\cdot$$7$H$_2$O</td>
<td>0.0290</td>
</tr>
<tr>
<td>CuSO$_4$$\cdot$5H$_2$O</td>
<td>0.0050</td>
</tr>
</tbody>
</table>

### Sigma® RPMI-1640 Vitamin Solution (100x)

<table>
<thead>
<tr>
<th>Components</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Biotin (C$<em>{10}$H$</em>{16}$N$_2$O$_3$S)</td>
<td>0.0200</td>
</tr>
<tr>
<td>Choline Chloride (C$<em>{5}$H$</em>{14}$ClNO)</td>
<td>0.3000</td>
</tr>
<tr>
<td>Folic Acid (C$<em>{19}$H$</em>{19}$N$_7$O$_6$)</td>
<td>0.1000</td>
</tr>
<tr>
<td>myo-Inositol (C$<em>6$H$</em>{12}$O$_6$)</td>
<td>3.5000</td>
</tr>
<tr>
<td>Niacinamide (C$_6$H$_6$N$_2$O)</td>
<td>0.1000</td>
</tr>
<tr>
<td>p-Amino Benzoic Acid (0.5Ca(H$_2$NC$_6$H$_4$CO$_2$Na))</td>
<td>0.1000</td>
</tr>
<tr>
<td>D-Pantothenic Acid ((C$<em>9$H$</em>{16}$NO$_5$)$_2$Ca)</td>
<td>0.0250</td>
</tr>
<tr>
<td>Pyridoxine$\times$HCl (C$<em>8$H$</em>{11}$NO$_3$)</td>
<td>0.1000</td>
</tr>
<tr>
<td>Riboflavin (C$<em>{17}$H$</em>{20}$N$_4$O$_6$)</td>
<td>0.0200</td>
</tr>
<tr>
<td>Thiamine$\times$HCl (C$<em>{12}$H$</em>{17}$ClN$_4$OS)</td>
<td>0.1000</td>
</tr>
<tr>
<td>Vitamin B-12 (Cobalamin)</td>
<td>0.0005</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2000</td>
</tr>
<tr>
<td>KH$_2$PO$_4$ (anhyd)</td>
<td>0.2000</td>
</tr>
<tr>
<td>NaCl</td>
<td>8.0000</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$(anyd)</td>
<td>1.1500</td>
</tr>
</tbody>
</table>
APPENDIX B

Aqueous Speciation of Uranium in Media: 10 μM initial U(VI) and 20 mM Organic Acid

The JCHESS algorithm was used in order to calculate the aqueous speciation of U in the experimental media and account for both organic and inorganic complexation of U(VI) (van der Lee and De Windt 1999). The database provided with JCHESS (van der Lee and De Windt 1999) along with U-ligand complexation stability constants from the literature (Smith et al. 2003 and Allard et al. 1980) and values corrected to infinite dilution (Morel and Hering 1993) when necessary were utilized for the calculations. The media composition was inputted into the algorithm (See 2.1 Bacteria Selection and Media Composition and Appendix A: Composition of Mineral Solution and Sigma® RPMI-1640 Vitamin Solution) and the extended Debye-Huckel model was utilized to account for ion activity coefficients. Some complexes have been removed from the figures for clarity purposes.
Figure 7: Uranyl-Lactate Speciation (10 µM initial U(VI) and 20 mM organic acid)

Figure 8: Uranyl-Pimelate Speciation (10 µM initial U(VI) and 20 mM organic acid)
Figure 9: Uranyl-Adipate Speciation (10 μM initial U(VI) and 20 mM organic acid)

Figure 10: Uranyl-Glutamate Speciation (10 μM initial U(VI) and 20 mM organic acid)
Figure 11: Uranyl-Succinate Speciation (10 µM initial U(VI) and 20 mM organic acid)

Figure 12: Uranyl-Malonate Speciation (10 µM initial U(VI) and 20 mM organic acid)
Figure 13: Uranyl-Oxalate Speciation (10 µM initial U(VI) and 20 mM organic acid)

Figure 14: Uranyl-Maleate Speciation (10 µM initial U(VI) and 20 mM organic acid)
Figure 15: Uranyl-Citrate Speciation (10 µM initial U(VI) and 20 mM organic acid)

Figure 16: Uranyl-Tiron Speciation (10 µM initial U(VI) and 20 mM organic acid)
Figure 17: Uranyl-NTA Speciation (10 µM initial U(VI) and 20 mM organic acid)

Figure 18: Uranyl-EDTA Speciation (10 µM initial U(VI) and 20 mM organic acid)
APPENDIX C

Aqueous Speciation of Uranium in Media: 100 µM initial U(VI) and 20 mM Organic Acid

The JCHESS algorithm was used in order to calculate the aqueous speciation of U in the experimental media and account for both organic and inorganic complexation of U(VI) (van der Lee and De Windt 1999). The database provided with JCHESS (van der Lee and De Windt 1999) along with U-ligand complexation stability constants from the literature (Smith et al. 2003 and Allard et al. 1980) and values corrected to infinite dilution (Morel and Hering 1993) when necessary were utilized for the calculations. The media composition was inputted into the algorithm (See 2.1 Bacteria Selection and Media Composition and Appendix A: Composition of Mineral Solution and Sigma® RPMI-1640 Vitamin Solution) and the extended Debye-Huckel model was utilized to account for ion activity coefficients. Some complexes have been removed from the figures for clarity purposes.
Figure 19: Uranyl-Lactate Speciation (100 µM initial U(VI) and 20 mM organic acid)

Figure 20: Uranyl-Pimelate Speciation (100 µM initial U(VI) and 20 mM organic acid)
Figure 21: Uranyl-Adipate Speciation (100 µM initial U(VI) and 20 mM organic acid)

Figure 22: Uranyl-Glutarate Speciation (100 µM initial U(VI) and 20 mM organic acid)
Figure 23: Uranyl-Succinate Speciation (100 µM initial U(VI) and 20 mM organic acid)

Figure 24: Uranyl-Malonate Speciation (100 µM initial U(VI) and 20 mM organic acid)
Figure 25: Uranyl-Oxalate Speciation (100 μM initial U(VI) and 20 mM organic acid)

Figure 26: Uranyl-Maleate Speciation (100 μM initial U(VI) and 20 mM maleic acid)
Figure 27: Uranyl-Citrate Speciation (100 µM initial U(VI) and 20 mM organic acid)

Figure 28: Uranyl-Tiron Speciation (100 µM initial U(VI) and 20 mM organic acid)
Figure 29: Uranyl-NTA Speciation (100 µM initial U(VI) and 20 mM organic acid)

Figure 30: Uranyl-EDTA Speciation (100 µM initial U(VI) and 20 mM organic acid)
APPENDIX D

Aqueous Speciation of Uranium in Media: 1 mM initial U(VI) and 200 mM Organic Acid

The JCHESS algorithm was used in order to calculate the aqueous speciation of U in the experimental media and account for both organic and inorganic complexation of U(VI) (van der Lee and De Windt 1999). The database provided with JCHESS (van der Lee and De Windt 1999) along with U-ligand complexation stability constants from the literature (Smith et al. 2003 and Allard et al. 1980) and values corrected to infinite dilution (Morel and Hering 1993) when necessary were utilized for the calculations. The media composition was inputted into the algorithm (See 2.1 Bacteria Selection and Media Composition and Appendix A: Composition of Mineral Solution and Sigma® RPMI-1640 Vitamin Solution) and the extended Debye-Huckel model was utilized to account for ion activity coefficients. Some complexes have been removed from the figures for clarity purposes.
Figure 31: Uranyl-Lactate Speciation (1 mM initial U(VI) and 200 mM organic acid)

Figure 32: Uranyl-Pimelate Speciation (1 mM initial U(VI) and 200 mM organic acid)
Figure 33: Uranyl-Adipate Speciation (1 mM initial U(VI) and 200 mM organic acid)

Figure 34: Uranyl-Glutarate Speciation (1 mM initial U(VI) and 200 mM organic acid)
Figure 35: Uranyl-Succinate Speciation (1 mM initial U(VI) and 200 mM organic acid)

Figure 36: Uranyl-Malonate Speciation (1 mM initial U(VI) and 200 mM organic acid)
Figure 37: Uranyl-Oxalate Speciation (1 mM initial U(VI) and 200 mM organic acid)

Figure 38: Uranyl-Maleate Speciation (1 mM initial U(VI) and 200 mM organic acid)
Figure 39: Uranyl-Citrate Speciation (1 mM initial U(VI) and 200 mM organic acid)

Figure 40: Uranyl-Tiron Speciation (1 mM initial U(VI) and 200 mM organic acid)
Figure 41: Uranyl-NTA Speciation (1 mM initial U(VI) and 200 mM organic acid)

Figure 42: Uranyl-EDTA Speciation (1 mM initial U(VI) and 200 mM organic acid)


accumulation by biofilm-immobilized and chemically-coupled cells of a *Citrobacter* sp. pre-grown in continuous culture. *Biotechnology and Bioengineering* **63**, 87-97


for Use in Chemical Barriers for Uranium Mill Tailings Remediation. 
*Environmental Science and Technology* 26, 1922-1931.


Tebo B.M. and 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.


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http://www.ne.doe.gov/uranium/facts.html

http://www.ocrwn.doe.gov/ym/index.shtml

US EPA (2005) NPL Database.
http://cfpub.epa.gov/supercpad/cursites/srchsites.cfm


