To the Graduate Council:

I am submitting herewith a thesis written by Lingling Chu entitled " Co-bioprecipitation of uranium and selected heavy metals in sulfate containing solution." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.

Gregory D. Reed, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and Dean of
The Graduate School

CO-BIOPRECIPITATION OF URANIUM AND SELECTED HEAVY METALS IN SULFATE CONTAINING SOLUTION

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Lingling Chu

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This thesis is dedicated to my mother

Mrs. He, Wenyuan

who expected this moment for a long time but has not had a chance to see it forever. Her encouragement has directed me to overcome all difficulties toward my degree.

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ABSTRACT

This study investigated several factors in the co-bioprecipitation of low concentration uranium and selected heavy metals in sulfate solution. The study also investigated the feasibility of the bio-metal removal process serving as a post-treatment method for the Department of Energy's process wastewater.

Desulfovibrio desulfuricans has the capability to biologically reduce soluble uranium (VI) to insoluble uranium (IV) and to concurrently precipitate toxic heavy metals with the produced sulfide. It was, therefore, chosen as the microorganism used in this study. Several selected factors that may affect this bio-metal removal process were examined in batch-scale experiments. These factors are: low initial concentration of uranium, sulfate concentration versus ionic strength, toxicity of heavy metals (zinc, nickel and copper), and chelating agents (citrate and EDTA). Synthetic wastewaters, which contained uranium, bicarbonate, lactate, sulfate, zinc, nickel, copper, citrate, and EDTA, were used for all experiments in the first phase of this study. Finally the more complex wastewater from the Y-12 Plant of the Department of Energy was evaluated for its biotreatability using this bio-metal removal process.

The experiments of the synthetic wastewater containing 4-6 mg/l of uranium demonstrated that uranium reduction by *Desulfovibrio desulfuricans* is possible at this low initial concentration. However, the uranium (VI) removal was found to be slower at this low initial concentration than that at the high initial concentration (100-200 mg/l).

Under the experimental conditions, the uranium reduction is approximately a first order reaction with a reduction rate constant of 0.3113 hr^{-1}.

As Sulfate concentration was gradually increased from 0 to 10,000 mg/l, the effectiveness of *D. desulfuricans* for uranium removal was observed to decrease. This decrease could be caused by a combined effect of high ionic strength, electron acceptor competition, and electron donor limitation. Furthermore, in a low ionic strength condition (experiments A and C), the effect of sulfate concentration appears to be more important than that of ionic strength. In a high ionic strength condition (experiments A and B), the effect of ionic strength appears to be more important than that of sulfate concentration.

Zinc and nickel were removed by indirect enzymatic sulfide precipitation at almost 100% efficiency within one hour and four hours, respectively. Since the bioreactors achieved higher metal removal efficiency than that in the abiotic metal-sulfide precipitation reactors under the experimental conditions, the bio-metal removal process may indeed have advantages over abiotic treatment systems. Copper was found to inhibit the bioactivity of *D. desulfuricans* at initial concentrations in excess of 1 mg/l.

Citrate and EDTA were chosen as reference ligands to evaluate the effect of ligands in a bicarbonate solution. Citrate was observed to partially affect uranium reduction and heavy metal removal. The metal-citrate stability constants were $10^{8.99}$ and $10^{6.76}$ for zinc and nickel respectively. These were smaller than the metal-bicarbonate stability constants of $10^{12.50}$ and $10^{12.60}$ for zinc and nickel respectively. EDTA on the other hand totally inhibited uranium reduction and heavy metal removal. The metal-

EDTA stability constants for these complexes were $10^{16.96}$ and $10^{20.85}$ for zinc and nickel respectively, and were larger than those of the metal-bicarbonate complexes.

For the given Y-12 Plant wastewater, the results demonstrate that, with appropriate control of the investigated factors, the bio-metal removal process using D. *desulfuricans* is a potentially effective post-treatment method for the Y-12 Plant and similar industrial wastewaters.

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CHAPTER I

INTRODUCTION

The production of uranium waste has increased significantly in the last decade, causing a high potential for uranium contamination of surface and groundwater. The mixed-waste stream from the Y-12 Plant, Oak Ridge, is one of these contaminant sources. This waste stream has been treated by wastewater treatment facilities. The treatment processes are oil/water separation, cyanide destruction, neutralization, biological denitrification, and biooxidation. The effluent still contains low concentrations of uranium and several other toxic heavy metals. Also numerous contaminants from the production process are in the wastewater, such as high concentrations of sulfate, nitrate and variable unknown organic chelating agents. Typical concentrations are: U(VI) 4-10 mg/l, zinc 10-12 mg/l, nickel 10-12 mg/l, copper 7-10 mg/l, bicarbonate 2,500 mg/l, sulfate 1,000-5,000 mg/l, nitrate 5,000 mg/l, and pH 7.0-8.0.

Uranium is a naturally occurring radionuclide. It has two major oxidation states; U(IV) and U(VI). U(IV) is normally found as the mineral uraninite (UO_2) which is insoluble. U(VI) is found in aqueous solutions as the uranyl ion (UO_2^{+2}) which is soluble (Allison et al., 1991). Since man-made radionuclide sources were added to those that occur naturally, through various exposure pathways, an average person living in the United States is apt to receive more radiation from man-made sources than from natural background (Turner, 1992). Depending on the dose, kind of radiation, and observed

endpoint, the damage of radiation on the human body can differ widely. Some occur relatively fast while others may take years to become evident, such as the damage of central nervous system appears within a matter of days or less; haemopoietic death occurs in about a month; cancer occurs many years after exposure to radiation; and a genetic effect occurs in the next or subsequent generations of an exposed individual (Turner, 1992). Because uranium species have a long half life (~10^9 years) and many uranium ions have a high valence, they have a higher potential to accumulate in the environment and the human body.

Other toxic heavy metals in the Y-12 Plant wastewater also have severe impacts on the environment and human health; for instance, a copper concentration less than 0.1 mg/l can inhibit the growth of most aquatic plants (Moore and Ramamoorthy, 1984). Although nickel is essential at trace levels for human health, it can induce nasal, laryngeal, and lung cancer when it combines with some other complexes (Pedersen et al., 1978; Lessard et al., 1978). Zinc may have toxicity to fish by occluding central blood spaces, decreasing blood pH, resulting in the dysfunction of kidney tissue and enzymes, and decreased growth, maximum size and fecundity (Moore and Ramamoorthy, 1984). Thus, The Resource Conservation and Recovery Act (RCRA) has enacted stringent regulations for their disposal.

In order to discharge the current Y-12 Plant process wastewater, a post treatment method is required to lower uranium and other toxic heavy metals concentrations. Since this waste stream has a complex chemistry, the metal species remain in solution even when the pH of the wastewater is raised to 10. Therefore, the conventional metal

treatment method, hydroxide precipitation, is not sufficient for metal removal from this wastewater. Other physical-chemical treatment technologies also have significant limitations for this complex wastewater.

Biological removal of toxic heavy metals is a potential alternative technique for this type of waste stream. According to the wastewater and organism characteristics, *Desulfovibrio desulfuricans* is the most suitable organism for this wastewater (see discussion below). Pure cultures of *Desulfovibrio* are commonly available. They are anaerobic organisms and their taxonomic character is dissimilatory sulfate reduction (Postgate, 1984), that is sulfate is used as a terminal electron acceptor rather than a nutrient or cell material. The sulfate is reduced to sulfide through their metabolism. This enzymatic sulfate reduction makes *D. desulfuricans* a sulfide producer in the wastewater whereby the sulfide could combine with toxic heavy metals to form metal sulfide precipitates. Metal sulfide precipitation is an effective method to remove metals from metal complex condition. In this process, the biological method is an indirect enzymatic metal removal mechanism since enzymatic bioreduction serves only as an indirect reaction to support heavy metal removal. The direct metal removal reaction is caused by sulfide precipitation. However, it has recently been proven that *D. desulfuricans* and some other organisms can also directly enzymatically reduce U(VI) to U(IV) in natural sediments (Lovley and Philips, 1991, 1992). In this process, the biological metal removal reaction is a direct metal removal mechanism. Therefore, *D. desulfuricans* has a potential ability to carry out direct and indirect enzymatic metal removal concurrently, facilitating the removal of uranium and other toxic heavy metals in the same system. If

this system could remove uranium and other heavy metals effectively, it would simplify the post treatment process and lower system installation and operation costs.

Most previous direct or indirect enzymatic metal removal studies utilizing *D. desulfuricans* have been conducted in relatively simple solutions, which used uranium or sulfate as the sole electron acceptors. They were conducted to investigate either direct enzymatic uranium reduction or indirect enzymatic metal removal. For the complicated situation, such as the Y-12 Plant wastewater, not only are two electron acceptors present in one system but also other heavy metals and chelating agents are present together. Sufficient information was not available to evaluate the potential effectiveness of this biological treatment method.

The objective of this study was to investigate the feasibility of employing the two mechanisms (direct enzymatic uranium reduction and indirect enzymatic metal removal) together in one biotreatment system. A pure culture of *D. desulfuricans* was used as a target organism throughout this study. Since bicarbonate was one of the components in the wastewater, all of the experiments were conducted in a bicarbonate-containing solution (2,500 mg/l). For comparison purposes, only lactate was used as an electron donor throughout this study.

The approach used to accomplish the research objective involved running two major bench scale experiments using synthetic wastewater and Y-12 Plant wastewater. The synthetic wastewater experiment investigated the impact of several factors separately, and Y-12 Plant wastewater was used to put the *D. desulfuricans* system in an actual situation. The result indicates that the *D. desulfuricans* biotreatment method is a

potential effective post-treatment technique for Y-12 Plant and similar waste streams. However, its application requires certain conditions.

CHAPTER II

LITERATURE REVIEW

The presence of radionuclides and toxic metals such as copper, iron, lead, nickel, cadmium, chromium, and zinc, in many waste streams from the Department of Energy (DOE) and other facilities is a major environmental concern (Macaskie, 1991; Ganesh, 1993). These waste streams include wastewaters that originate from radioactive plants, nuclear fuel, or are produced from reactor operations and fuel reprocessing. In order to prevent environmental pollution, this mixed-waste stream requires stringent treatment under Resource Conservation and Recovery Act (RCRA) before discharge. Both metal and radionuclide contaminants must be removed from the waste stream to reach RCRA goals.

2.1. Uranium removal

Uranium waste generated from Y-12 Plant facilities could be removed from contaminated water by technologies such as coagulation/filtration, reverse osmosis, or ion exchange (Kucsmas, 1994). However, the desirability of these technologies are limited by the material or disposal cost, the interference caused by pH or competing ions, and the original uranium concentration (Lovley and Phillips, 1992). Recently, geologists have recognized that microbial uranium reduction by anaerobic microorganisms plays a potentially important role in the formation of ore deposits in sediments (Lovley et al.,

1989). Since this method may provide a cost-effective mechanism for uranium removal, it is receiving increased attention as a treatment alternative.

2.1.1 Microbial uranium reduction

Microbial uranium reduction is an important process in the global uranium cycle and may also be a useful technique for removing uranium from contaminated wastes. It is a mechanism that can be carried out by three kinds of organisms: Fe(III)-reducing organisms (*Geobacter metallireducens* (formerly strain GS-15), *Shewanella* (formerly *Alteromonas*) *putrefaciens*), sulfate-reducing organisms (*Desulfovibrio desulfuricans*) (Lovley and Phillips, 1992), and denitrification organisms (*Pseudomonas denitrificans*) (Thomson et al., 1994). These organisms have a common ability to enzymatically reduce U(VI) (oxidized form) to U(IV) (reduced form). The oxidized form U(VI) is typically a highly soluble uranium form, and the reduced form U(IV) is a highly insoluble uranium form. Therefore, uranium could be separated from the wastewater by this mechanism.

Geobacter metallireducens (GS-15) is the Fe(III)-reducing organism. They grow under anaerobic conditions by enzymatically coupling the oxidation of acetate to carbon dioxide with the reduction of Fe(III) to Fe(II) (Lovley et al., 1987; Lovley and Phillips, 1988). Lovley and his co-worker (1991) inoculated GS-15 into an anaerobic medium with acetate as the sole electron donor and U(VI) as the potential electron acceptor to investigate whether GS-15 could directly enzymatically reduce U(VI) to U(IV). Their results showed that U(VI) was reduced to U(IV) from 8.0 mmol/l to 2.2 mmol/l within 25 hours, and also cell growth coincided with U(VI) reduction and stopped as U(VI) became depleted (the cell number increased from 3.0×10^7/ml to 8.5×10^7/ml within 25 hours).

This indicated that GS-15 can obtain energy for growth by oxidizing acetate with the reduction of U(VI) to U(IV). The potential reduction reaction is shown as follows:

$$CH3COO^- + 4U(VI) + 4H_2O \rightarrow 4U(IV) + 2HCO_3^- + 9H^+$$

Alteromonas putrefaciens is also the Fe(III)-reducing organism. In Lovley's research (1991), the organism was grown with H_2 as the sole electron donor and U(VI) as the electron acceptor. The results indicated that *A. putrefaciens* could rapidly enzymatically reduce U(VI) to U(IV) according to following reaction:

$$H_2 + U(VI) \rightarrow U(IV) + 2H^+$$

U(VI) decreased from 7 mmol/l to 1.5 mmol/l, and the cell number increased from 0.5×10^7/ml to 4×10^7/ml within 30 hours. Other electron donors for Fe(III) reduction by *A. putrefaciens* (formate, lactate, pyruvate) also supported U(VI) reduction.

Desulfovibrio desulfuricans is the sulfate-reducing organism. It was also demonstrated as a contributor to U(VI) reduction in sedimentary environments (Lovley and Phillips, 1992). U(VI) reduction with lactate or H_2 as the electron donor in cell suspensions of *D. desulfuricans* resulted in the formation of a black precipitate. According to X-ray diffraction analysis, this black precipitate was made up of the U(IV)-containing mineral uraninite (UO_2). In addition, the transmission electron microscopy indicated that the uranium precipitation was extracellular in the cell suspension. From

comparison experiments, it was indicated that *D. desulfuricans* reduced U(VI) faster than GS-15 and more slowly than *A. putrefaciens*. The potential reaction of U(VI) reduced to U(IV) using lactate as the electron donor is as follows (Kucsmas, 1994):

$$UO_2^{+2} + C_3H_6O_3 \rightarrow UO_2 + C_2H_4O_2 + 2H^+ + CO_2$$

However, it was found that uranium reduction could not support *D. desulfuricans* growth.

Among the three organisms above, *D. desulfuricans* is the most valuable one for bioremediating a uranium-contaminated waste stream. The reasons are:

1. *D. desulfuricans* is easier to culture than other obligatory anaerobic bacteria; even freeze-dried cells kept under air lost none of their potential for U(VI) reduction after six months of storage (Lovley and Phillips, 1992; Krieg and Holt, 1984).

2. The U(VI)-reducing capacity of *D. desulfuricans* is very stable at both high or low uranium concentration (Lovley, 1993).

3. *D. desulfuricans* has characteristics of both fresh water and marine organisms (Krieg and Holt, 1984). It can tolerate high ionic strength in the water as in the ocean, as well as live in low concentration of NaCl, as in fresh water. This is a suitable characteristic for surviving in a variety of industrial waste streams.

These three advantages make microbial uranium reduction easier to apply in a wide range of conditions, from diluted groundwater, surface water, or leachate to concentrated industrial waste streams. Therefore, the research presented here chose *D. desulfuricans* as the treatment organism for the bioreactor experiments.

2.1.2. *Desulfovibrio Desulfuricans*

D. desulfuricans belongs to the sulfate-reducing bacteria family. Sulfate-reducing bacteria are important organisms in many anaerobic environments in nature. They belong to a distinctive and ubiquitous group of anaerobic prokaryotes which are unified by a shared ability to carry out sulfate reduction as a principal component of their bioenergetic processes. The principal component is that the sulfur atom in the sulfate ion, which has a valence +6, is reduced to -2 to form the sulfide ion, S^{2-}. The chemical reaction is shown as follows (Odom and Singleton, 1993):

$$4\ AH_2 + SO_4^{2-} + H^+ \rightarrow 4\ A + HS^- + 4\ H_2O$$

Electron donor Sulfate-reducing bacteria can use a variety of electron donors (AH_2) for their catabolic and anabolic metabolism. Most of the compounds that have been shown to be utilized as electron donors by sulfate-reducing bacteria are listed in Table 1 (Odom and Singleton, 1993).

Some of these compounds are used by many strains, whereas others have been identified as a suitable energy source for only a single pure culture. Two major metabolic groups of sulfate-reducing bacteria may be distinguished. Group (A) comprises species oxidizing their electron donor incompletely to acetate which cannot be oxidized further. Group (B) embraces the sulfate-reducing bacteria which are, in principle, able to oxidize their electron donor, including acetate, completely to carbon dioxide. *D. desulfuricans*

Table 1. Compounds that can be used as electron donors for dissimilatory sulfate reduction by sulfate-reducing bacteria

Class of Compound	Specific Compounds Utilized
Inorganic	Hydrogen, carbon monoxide
Hydrocarbons	Straight alkanes (C_{12} to C_{20})
Monocarboxylic acids (aliphatic)	Formate, acetate, propionate, butyrate, higher fatty acids up to C_{20}, isobutyrate, 2-methylbutyrate, 3-methylbutyrate, 3-methylvalerate, pyruvate, lactate
Dicarboxylic acids	Succinate, fumarate, malate, oxalate, malonate, glutarate pimelate
Alcohols	Methanol, ethanol, propanol-1, butanol-1, pentanol-1, isobutanol, propanol-2, butanol-2, ethylene glycol (mono-, di-, tri- and tetra-), 1-,2-propanediol, 1,3-propanediol, glycerol
Amino acids	Glycine, serine, alanine, cysteine, cystine, threonine, valine, leucine, isoleucine, aspartate, glutamate, phenylalanine
Sugars	Fructose, glucose, mannose, xylose, rhamnose
Aromatic compounds	Benzoate, 2-, 3-, and 4-hydroxybenzoate, phenol, p-cresol, catechol, resorcinol, hydroquinone, phloroglucinol, pyrogallol, 2-,4-dihydroxybenzoate, 3-,4-dihydroxybenzoate (protocatechuate), 2-aminobenzoate (anthranilate), 4-aminobenzoate, aminobenzene (aniline), hippurate, nicotinic acid, indole, quinoline, phenylacetate, 4-hydroxyphenylacetate, indolylacetate, 3-phenylpropionate,[a] trimethoxybenzoate,[a] *m*-anisate,[a] *p*-anisate,[a] vanillate,[a] 3-methoxysalicylate,[a] vanillin,[a] syringaldehyde,[a] *p*-anisaldehyde,[a] 2- and 3-hydroxybenzaldehyde[a]
Miscellaneous	Choline, betaine, oxamate, acetone, dihydroxyacetone, furfural, cyclohexanone, cyclohexanol, cyclohexanecarboxylate and a few other alicyclic compounds

Source: Odom, J. M. and Singleton, R. Jr., 1993. The Sulfate-reducing Bacteria: Contemporary Perspectives, p. 22

may belong to group (A), since they can not use acetate as the electron donor (Krieg and Holt, 1984). The oxidation-reduction reactions for different electron donors in a sulfate-reducing bacteria's metabolism are showed as follows (Zehnder,1988):

Lactate: $2\ CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + HS^- + H^+$

$\Delta G^0 = -160.1$ kj

$2\ CH_3CHOHCOO^- + 3\ SO4^{2-} \rightarrow 6\ HCO_3^- + 3HS^- + H^+$

$\Delta G^0 = -225.3$ kj

Acetate: $CH_3COO^- + SO_4^{2-} \rightarrow 2\ HCO_3^- + HS^-$

$\Delta G^0 = -47.6$ kj

Propionate: $4CH_3CH_2COO^- + 3\ SO_4^{2-} \rightarrow 4\ CH_3COO^- + 4\ HCO_3^- + 3\ HS^- + H^+$

$\Delta G^0 = -150.6$ kj

For *D. desulfuricans*, the electron donors are restricted to lactate, pyruvate, formate, choline, or certain simple primary alcohols including methanol, ethanol, propanol and butanol (Krieg and Holt, 1984).

Electron acceptor The common terminal electron acceptors that the sulfate-reducing bacteria use in their metabolism are sulfate or other sulfur compounds. However, *D. desulfuricans* has been found to have one of the most versatile respiratory metabolisms known (Dilling and Cypionka, 1990). They use not only sulfate, but also

O_2, NO_3^-, Fe(III), and U(VI) as electron acceptors for electron transport (Dalsgaard and Bak, 1994; Coleman, et al., 1993; Lovley and Phillips, 1992). The possible enzymatic reduction reactions are:

$$4\ Fe_2O_3 + CH_3COO^- + 7\ H_2O \rightarrow 8\ Fe^{2+} + 2\ HCO_3^- + 15\ OH^-$$

$$Fe_2O_3 + H_2 + H_2O \rightarrow 2Fe^{2+} + 4\ OH^-$$

$$2\ NO_3^- + C_3H_6O_3 \rightarrow C_2H_4O_2 + CO_2 + 2NO_2^- + H_2O$$

$$UO_2^{+2} + C_3H_6O_3 \rightarrow UO_2 + C_2H_4O_2 + 2H^+ + CO_2$$

This electron acceptor versatility makes concurrent reduction of sulfate and uranium possible in natural aquatic sediments and in bioreactors.

Energy metabolism Respiratory sulfate reduction involves a minimum of four cytoplasmic enzymes (Odom and Peck, 1984): ATP sulfurylase; inorganic pyrophosphatase; adenylyl sulfate (APS) reductase; and bisulfite reductases. Their energetic conversion is shown as follows:

$ATP + SO_4^{2-} \leftrightarrow APS + PP_i$ ATP sufurylase

$PP_i + H_2O \rightarrow 2P_i$ inorganic pyrophosphatase

$APS + H^+ + 2e \leftrightarrow HSO_3^- + AMP$ APS reductase

$HSO_3^- + 6H^+ + 6e \rightarrow HS^- + 3\ H_2O$ bisulfite reductase

APS reductase is an enzyme which catalyzes the reversible reduction of APS to sulfite plus AMP (Peck and Le Gall, 1982). The reductase also occurs in some phototrophic and sulfur-oxidizing bacteria capable of oxidizing reduced sulfur compounds to sulfate and results in a substrate-level conservation of energy. Neither $NADH_2$ nor $NADPH_2$ directly functions as the electron donor for APS reductase, and the in vivo electron donor for this reduction has not been established, but the electron transfer appears to involve membranes (Kremer and Hansen, 1989). APS reductase is believed to be an indicator for the presence of sulfate-reducing bacteria.

Bisulfite reductases have four different molecular species: desulfoviridin; desulforubidin; P_{590}; and desulfofucidin. They are distinguished from nitrite and other sulfite reductase by their ability to catalyze the irreversible formation of trithionate and thiosulfate under certain assay conditions, but the role of these reactions in the pathway of respiratory sulfate reduction has not yet been clearly resolved (Odom and Singleton, 1993). Neither $NADH_2$ nor $NADPH_2$ directly functions as an electron donor for bisulfite reductase and, as is the case for APS reductase, the in vivo electron donor for the six-electron reduction has not been established.

According to the energetic conversion above, the reaction of sulfate consumes 1 molecule ATP per sulfate ion and yields 1 molecule of AMP, two molecules of acetyl phosphate would be needed to regenerate a molecule of ATP from that AMP. If lactate is used to obtain those two acetyl phosphate ions, an ATP balance of bacterial sulfate

reduction is shown in Figure 1 (Postgate, 1984). One molecule of ATP is generated from lactate at the substrate level and one is consumed at the sulfate reduction level.

2.2. Heavy metal removal

The Y-12 Plant wastewater, as well as other radionuclide-bearing waste streams, not only contains radiotoxic metal (uranium), but also contains several other heavy metals, such as zinc, nickel and copper, that should be removed. The common treatment methods for removing heavy metals from a wastewater are chemical precipitation and microbial metal removal.

2.2.1. Chemical precipitation

The basic process of chemical precipitation is adding a chemical reactant to the metal-contaminated wastewater. The dissolved metals are converted to an insoluble form by a chemical reaction between the soluble metal compounds and reactant. The insoluble metal precipitates are usually separated from the wastewater by a physical settling process.

Hydroxide precipitation Hydroxide precipitation involves the use of calcium hydroxide (lime) or sodium hydroxide (caustic) as the reactant to remove metals as insoluble metal hydroxides. The reaction is illustrated by the following equation:

$$M^{2+} + Ca(OH)_2 \rightarrow M(OH)_2 + Ca^{2+}$$

The metal removal efficiency is dependent on the metal present, the reactant used, the reaction conditions (especially pH), and the presence of other chemical compounds that

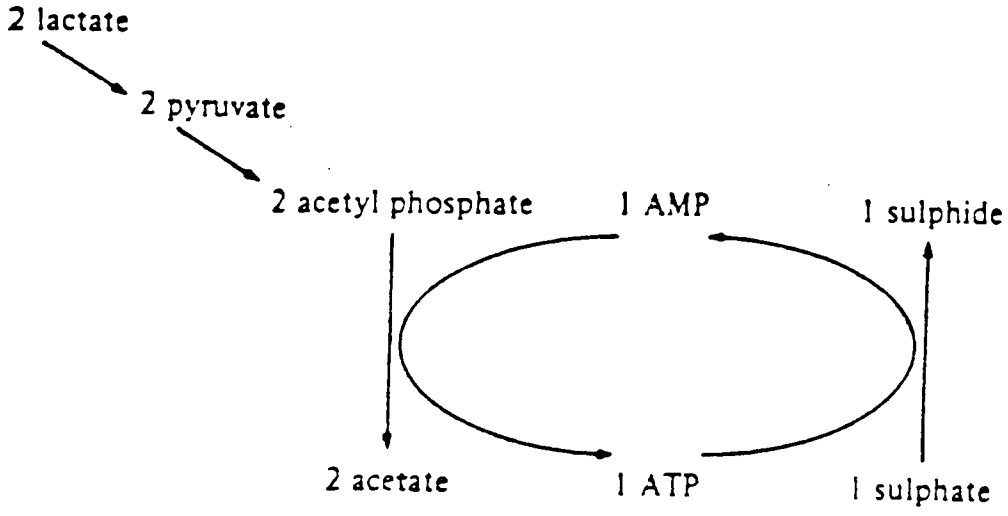

Figure 1. The ATP balance of bacterial sulfate reduction

Source: Postgate, J. R. 1984, The Sulfate-reducing Bacteria (2nd). P. 92.

may inhibit precipitation. The theoretical solubility of several metal hydroxides are shown in Figure 2 (Freeman, 1989).

Figure 2 indicates that metal hydroxides are amphoteric. Their solubilities increase at both low and high pH. And the optimum pH for precipitation occurs at different pH values for different metals. So at a pH at which the solubility of one metal hydroxide may be minimized, the solubility of another may be relatively high. According to jar tests and operating experience with waste streams, a pH between 9 and 11 usually produces acceptable effluent quality. For a waste that contains several metals, however, more than one precipitation stage with different pH control points may be required to remove all metals of concern to desired levels. Patterson et al. (1977) observed that hydroxide precipitation, although very effective, has some inherent problems. These include poor dewaterability of the sludge due to the gelatinous nature of the sludge, large volumes of sludge formed, and in the case of some metals (such as nickel) which have an optimum pH above 10, continuous pH adjustment which increases the overall treatment cost.

Sulfide precipitation The sulfide precipitation process is effective for heavy metal removal. The precipitation of a dissolved metal ion as a metal sulfide (MS) occurs when the metal ion (M^{2+}) contacts a sulfide ion (S^{2-}):

$$M^{2+} + S^{2-} \rightarrow MS \downarrow$$

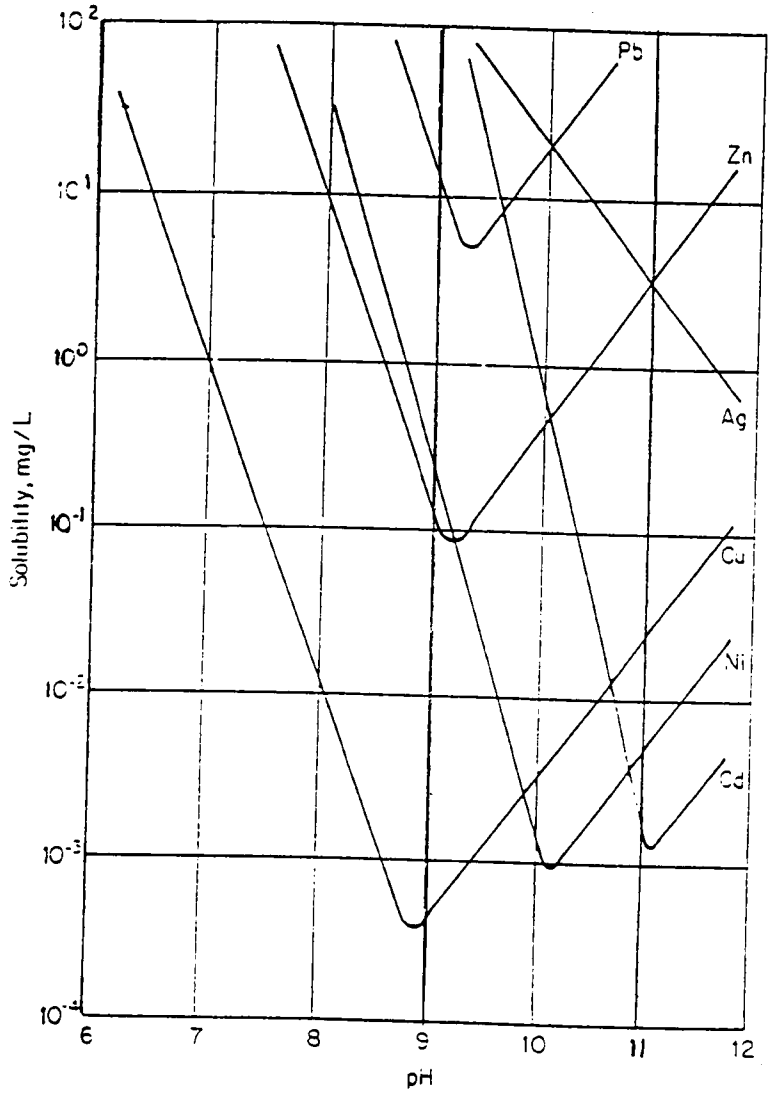

Figure 2. Solubilities of metal hydroxides as a function of pH.

Source: Freeman, H. M., 1989. Standard Handbook of Hazardous Waste Treatment and Disposal. p. 7.24

Table 2 (Weast, 1969) lists the important solubility product constants (Ksp) for some metal sulfides and Figure 3 (Freeman, 1989) shows solubility of metal sulfides. Table 2 shows that almost all metal-sulfide compounds have very low solubility. If the concentration product for metal and sulfide ions in a wastewater is higher than the solubility constant, metal sulfide could be precipitated from the solution until the solution reaches chemical equilibrium. If there are enough sulfide ions in the solution to combine with metal ions, most metal ions would likely be converted to highly insoluble metal sulfides, then separated from the wastewater.

The sulfide precipitation process has its advantages over hydroxide precipitation. The attractive features include (Blythe et al., 1981):

- A high degree of metal removal over a broad pH range.
- Effective precipitation of metals, such as As, Cd, and Hg, even at a very low pH.
- Low retention time required in the reaction tank because of the high reactivity of sulfides.
- The feasibility of selective metal recovery.
- Effective removal of many complexed heavy metals.

All these features make sulfide precipitation a valuable process now and in the future for decontaminating industrial wastewaters that contain toxic heavy metals. There are two processes that are currently employed to precipitate metals as sulfides that differ mainly in the method used to introduce the sulfide ions into the wastewater (I. E. R. L., 1980).

Table 2. Solubility of sulfides

Metal sulfide	K_{sp} (64° to 77° F)	Sulfide concentration (mol/l)
Manganous sulfide	1.4×10^{-15}	3.7×10^{-8}
Ferrous sulfide	3.7×10^{-19}	6.1×10^{-10}
Zinc sulfide	1.2×10^{-23}	3.5×10^{-12}
Nickel sulfide	1.4×10^{-24}	1.2×10^{-12}
Stannous sulfide	1.0×10^{-25}	3.2×10^{-13}
Cobalt sulfide	3.0×10^{-26}	1.7×10^{-13}
Lead sulfide	3.4×10^{-28}	1.8×10^{-14}
Cadmium sulfide	3.6×10^{-29}	6.0×10^{-15}
Silver sulfide	1.6×10^{-49}	3.4×10^{-17}
Bismuth sulfide	1.0×10^{-97}	4.8×10^{-20}
Copper sulfide	8.5×10^{-45}	9.2×10^{-23}
Mercuric sulfide	2.0×10^{-49}	4.5×10^{-25}

Source: Weast, R. C., 1969. Handbook of Chemistry and Physics, 50th ed. p. B252

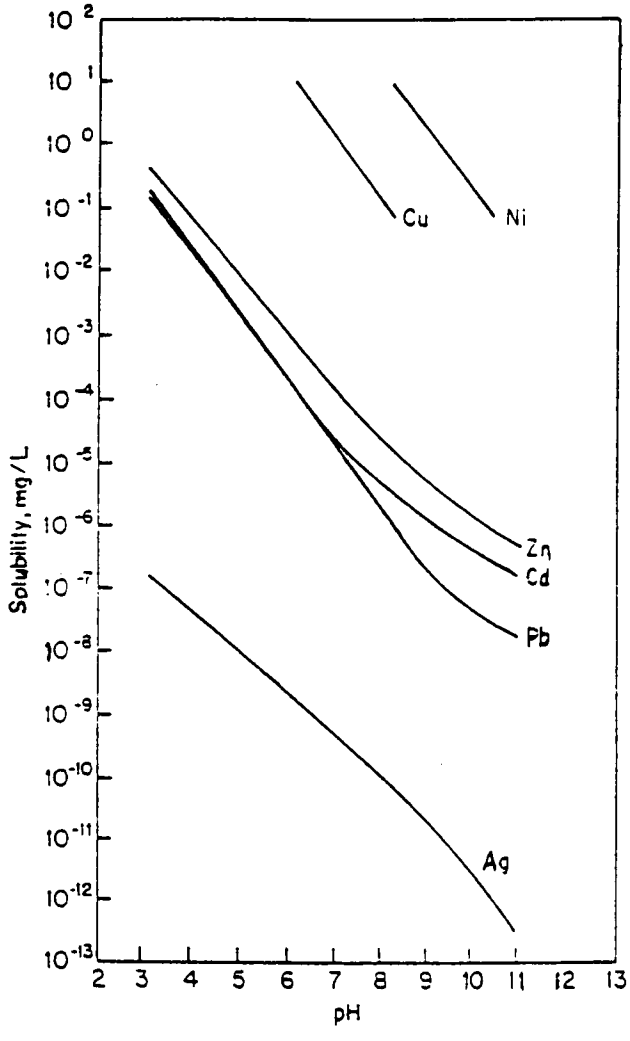

Figure 3. Solubilities of metal sulfides as a function of pH.

Source: Freeman, H. M., 1989. Standard Handbook of Hazardous Waste Treatment and Disposal. p. 7.25

The first process is soluble sulfide precipitation (SSP). In this process, the sulfide is added in the form of a water-soluble sulfide reagent such as sodium sulfide (Na_2S) or sodium hydrosulfide (NaHS). These sulfide reagents with the high solubility in the wastewater will yield a relatively high concentration of dissolved sulfide, which causes a rapid precipitation of the dissolved metals in the water as metal sulfides. The SSP process, however, also has a disadvantage. According to colloid and surface chemistry, there are two distinct stages in the process of a new phase formation during precipitation: nucleation and crystal growth (Shaw, 1991). If the nucleation stage is faster than the crystal growth stage, the small dispersed particles could form more easily than large particle flocs. If the crystal growth stage is faster than the nucleation stage, more large particles may be present in the solution. In the SSP process, the rapid precipitation of metal sulfide often results in the generation of small particle fines and hydrated colloidal particles. This situation tends more toward the nucleation stage than toward the crystal growth stage, which makes separating the metal-sulfide particles from the wastewater difficult. This process, therefore, needs the effective use (separately or combined) of coagulants and flocculants to aid in the formation of large particle flocs for efficient settling.

The second process used to precipitate metals as sulfides is the insoluble sulfide precipitation process (ISP). This process has an important advantage over the SSP process. In the ISP process, the sulfide is added in the form of a slightly soluble ferrous sulfide (FeS) slurry instead of a soluble sulfide reagent. The wastewater containing heavy metal is mixed with the FeS slurry in a solid/liquid contact chamber. The FeS gradually

dissolves to maintain the sulfide ion concentration at a certain level to prevent the nucleation stage from progressing faster than the crystal growth stage. Then the metal sulfide can be easily separated from the wastewater. Another advantage of the ISP process is the ability of the sulfide and ferrous ions to reduce hexavalent chromium to its trivalent state, which eliminates the need to segregate and treat chromium wastes separately. Under alkaline condition, the chromium will then precipitate as chromium hydroxide [$Cr(OH)_3$]. The overall reduction reaction is represented in the following formula (I. E. R. L., 1980):

$$H_2CrO_4 + FeS + 4\ H_2O \rightarrow Cr(OH)_3 \downarrow + Fe(OH)_3 \downarrow + S \downarrow + 2\ H_2O$$

Scott (1993) summarized the use of a sodium sulfide/ferrous sulfide metal precipitation process on electroplating wastewater at Tyndall and Sheppard Air Force Bases. The metals of interest were chromium, cadmium, copper, lead, zinc, nickel, and aluminum. The heavy metal levels of the effluent remained well below NPDES regulation limits. Yeligar et al. (1981) conducted a full scale ISP process for a metal-finishing wastewater from Holley Carburetor Division Colt Industries. This wastewater contained various materials which came from metal finishing operations, such as zinc, hexavalent chromium, aluminum, ortho- and polyphosphates, silicates, organic surfactants and chelants. The results demonstrated that the ISP process achieved metal values close to or less than the detection limit of the accepted analytical methods (i.e. Cr and Fe detected at 0.05 mg/l, Cu and Zn detected at 0.01 mg/l). The results also

demonstrated that ISP process produced more complete metal removal and acceptable effluent quality than the conventional hydroxide treatment of metal finishing wastewater. Also, several other researchers demonstrated that sulfide precipitation is an effective and economic treatment process for heavy metal contaminated wastewater (Shlauch, 1977; US A. B. R. D.C., 1984; I. E. R. L. 1979). However, the metal-sulfide precipitation system also has disadvantages that should be considered. First, there is a potential of generating toxic hydrogen sulfide gas (H_2S), which could contaminate the work area and surrounding environment. To prevent this problem, care must be taken to maintain the pH above 8 to prevent evolution of hydrogen sulfide. Even at an alkaline pH level, hydrogen sulfide may be emitted through rapid hydrolysis where a soluble sulfide salt is added to the water. Second, excessive sulfide may be released in the effluent. Sulfide is toxic to fish, can exert oxygen demand, and can cause a serious odor problem (Singh, 1992). To minimize this problem, the soluble sulfide salt should be added in a closed system with adequate ventilation, with the sulfide dosage carefully controlled by the equipment to avoid excess (Freeman, 1989).

2.2.2. Microbial metal removal

Microbial metal removal is another treatment method for the removal of heavy metal and radionuclides from a wastewater. Gadd (1988) summarized several microbial mechanisms which exist:

Biosorption Biosorption is a term used to describe the non-directed physico-chemical interactions that may occur between metal species and cellular components (Shumate and Strandberg, 1985). It can occur on both living cells and dead cells.

Biosorption involves the accumulation or concentration of metals on the organism cell wall. It is caused by the attraction of positively-charged ions to negatively-charged ligands in cell material (Brierley and Brierley, 1983), Van der Waals forces between cells and substances, or chemical attraction between cells and substances.

Intracellular accumulation Intracellular uptake of metal ions by organisms may be a slower process than biosorption. Many metals are essential for organism growth and metabolism, such as Cu, Fe, Zn, and Co. The organism's process transport system has varying specificity for metals accumulation from the external environment. Non-essential metal may also be taken via such systems. For example, cadmium appears to enter via a manganese system in several bacteria, algae, and fungi. The transport mechanisms are still uncertain (Gadd, 1988).

Extracellular precipitation Many bacteria produce large amounts of extracellular polymers that form capsules or loose aggregates around cells. These polymers often have anionic properties and are capable of binding with metal cations (Lester et al., 1984). Also dissimilatory sulfate-reducing bacteria can release sulfide, which in turn combines with metal ions to form metal sulfide which could accumulate on cell surfaces (Kelly et al., 1979). This indirect enzymatic metal-sulfide precipitation mechanism uses sulfate-reducing bacteria as a sulfide ion source. Therefore, enzymatic sulfate reduction only serves as an indirect metal removal mechanism. The direct metal removal is caused by chemical sulfide precipitation. Several researches have demonstrated that sulfate-reducing bacteria can effectively remove heavy metals from wastewaters by this indirect enzymatic metal-sulfide precipitation (P. E., 1991; Wijaya et

al., 1993; Singh, 1992). This mechanism is also one of the basic mechanisms for this study.

Bio-metal transformation Some organisms can transform the original chemical form of metals into other chemical form which is less toxic than the original one by enzymatic reduction, oxidation, methylation, demethylation, etc (Wood and Wang, 1983). The enzymatic uranium reduction discussed above belongs to this type of mechanism.

2.3. Effecting factors for both direct enzymatic uranium reduction and indirect enzymatic metal-sulfide precipitation.

Okabe and Characklis (1992) investigated the effects of temperature and phosphorous concentration on the rate and extent of microbial sulfate reduction with lactate as the electron donor at pH 7.0 for *D. desulfuricans*. Figure 4 shows that the maximum specific growth rate, μ_{max}, was relatively constant in the range 25°C to 43°C and dramatically decreased outside this temperature range. Also cell yield was highest in the optimum temperature range of 35°C to 43°C for growth. The limiting C : P radio was in the range of 400:1 to 800:1.

Gorby and Lovley (1992) also found that uranium reduction by *D. desulfuricans* occurred at 30°C, but not 4°C. Their results are shown in Figure 5.

Lovley and Phillips (1992) conducted experiments to investigate ion inhibition for uranium reduction by *D. desulfuricans*. Nitrate, molybdate, vanadyl, vanadate, sulfate, selenate, Mn(II), Ni(II), Fe(II), Cu(II), Cu(I), Mg(II), Zn(II), and Co(II) were added to the bioreactor separately. The results indicated that copper is the only one likely to inhibit microbial uranium reduction. Thomson et al. (1994) studied the effect of V, As, Se, and

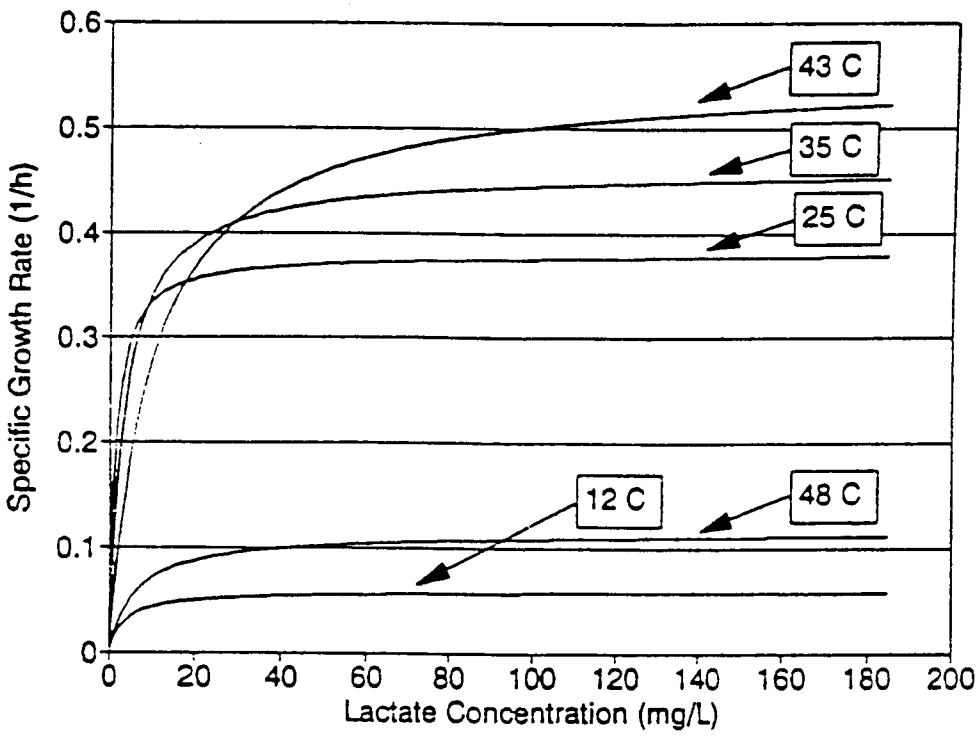

Figure 4. Kinetic parameters of *D.desulfuricans* growth on lactate as a function of temperature.

Source: Okabe, S. and Characklis W. G., 1992. Effects of Temperature and Phosphorous Concentration on Microbial Sulfate Reduction by *Desulfovibrio desulfuricans.* Biotechnology and Bioengineering. Vol. 39, p. 1040

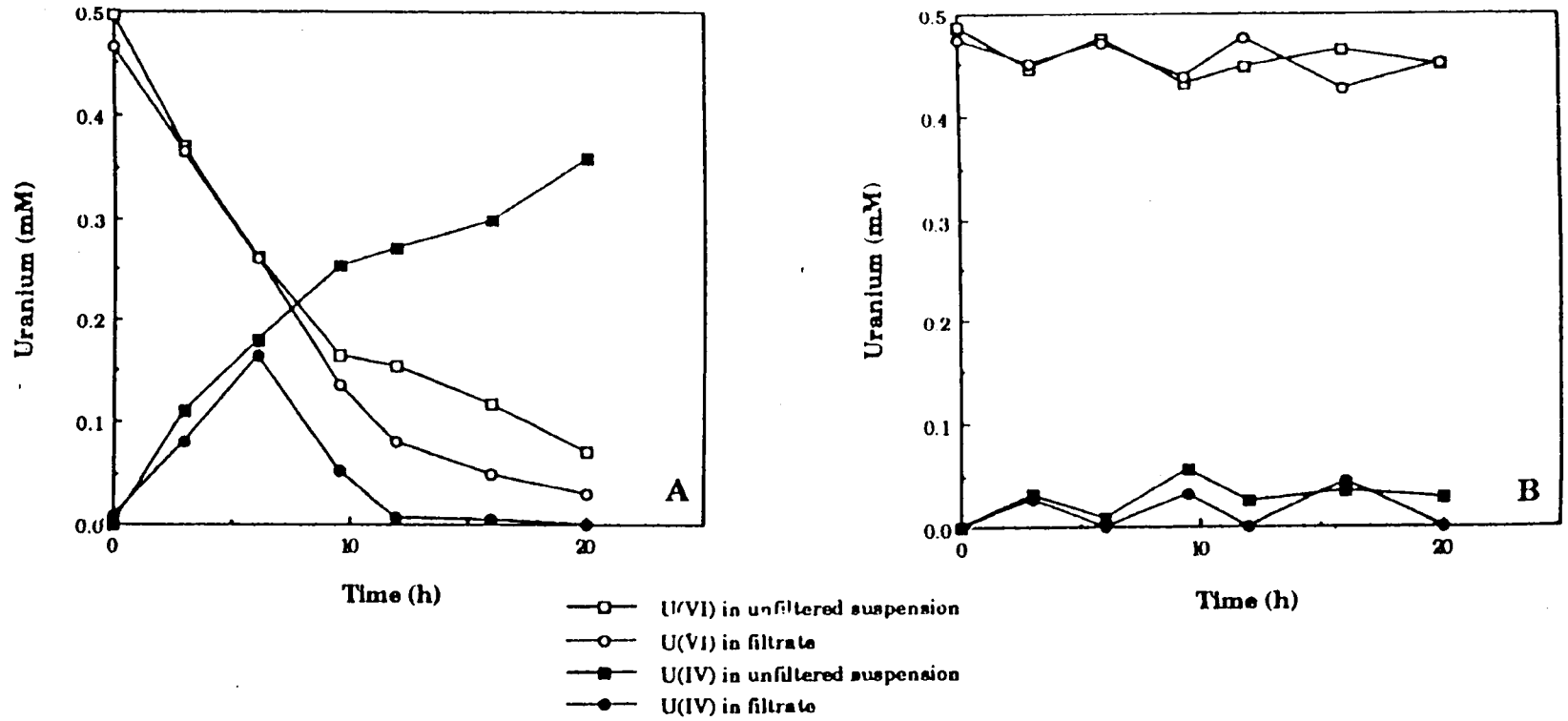

Figure 5. Time course of uranium reduction and precipitation at 30 (A) and 4 °C (B)

Source: Gorby, Y. A. and Lovley, D. R., 1992. Enzymatic Uranium Precipitation. Environ. Sci. Technol. 1992, 26, p. 206.

Mo for uranium reduction. They used several different U(VI) reducers (including *D. desulfuricans*) for their study. None of ions above produced inhibition to uranium reduction by either group of bacteria. Kucsmas (1994) evaluated the effect of nitrate and sulfate concentration on uranium reduction by *D. desulfuricans*. In his experiments (initial U(VI) concentration was around 100 mg/l), lower concentrations did not show inhibition, but concentrations of 50,000 mg/l nitrate or 10,000 mg/l sulfate might cause decline of uranium reduction rate.

Inorganic and organic chelating agents in the solution are also important effecting factors for both enzymatic uranium reduction and metal sulfide precipitation. Since organic chemicals are used for radioactive processing or nuclear decontamination operations (Macaskie, 1991), there are organic chelating agents in radionuclide waste streams. Organic chelating agents affect enzymatic uranium reduction and metal sulfide precipitation by several factors:

- The toxicity of metal complex to the organisms
- The stability constant of uranium or metal complex
- The species of the metal complex
- The structure of the metal complex
- The pH of the solution

Madsen and Alexander (1985) investigated the biodegradability of various metal-oxalate complexes, metal-citrate complexes, metal-NTA complexes and metal-EDTA complexes by sewage and other organisms. Their results showed that sewage organisms

biodegraded calcium citrate more rapidly than iron, aluminum, and magnesium citrate. The aluminum and iron oxalate were biodegraded more rapidly than calcium and magnesium oxalate. Sewage organisms could biodegrade calcium NTA complex, but not aluminum, magnesium, or iron NTA, or any of the EDTA complexes. However, in order to test whether the different biodegradabilities of these organism were caused by toxicity of the metal complex, they added glucose to the metal complex solutions. They found that glucose was biodegraded at identical rates in four of five organism solutions; only one case showed that the glucose degradation rate was delayed. This result indicated that the differences in biodegradation among four of five bacteria tested were not caused by metal complex toxicity.

Francis et al. (1992) studied the biodegradability of various structures of metal citrate complex by several organisms. They found that the type of complex formed between the metal and citrate is important in determining its biodegradability. For example, Ca, Fe(III), and Ni formed mononuclear bidentate complexes and were readily biodegraded, whereas Ca, Cu, Fe(II) and Pb, which formed mononuclear tridentate complexes, and U, which formed a binuclear complex involving the hydroxyl group of the citrate, were not biodegraded. These researchers also added glucose to the solutions to demonstrate that the metal complexes were not toxic to the organisms.

Complexation also affects the solubility of a metal sulfide. When any of the constituent ions of a solid participate in complex formation following dissolution, there will be an increase in the solubility of the solid (Snoeyink and Jenkins, 1980). This effect

is based on the stability constant of the metal complex and the solubility product of the solid precipitate. The equations for these two parameters are shown as follows:

$M + S \leftrightarrow MS \downarrow$ solubility product = [M] [S]

$M + L \leftrightarrow ML$ stability constant = [ML]/[M] [L]

Where, M: Metal ion;

S: Sulfide ion;

L: Organic ligand.

According to chemical equilibrium, in a metal sulfide precipitation system, if some organic ligands are present, the stronger the organic ligand, the more difficult the metal sulfide precipitation, since metal forms strong complexes with the organic ligands, which greatly decreases the amount of free metal ion in the solution for metal sulfide precipitation processes. However, since most metal sulfides have lower solubility products than metal hydroxides, they are relatively more insensitive to the presence of most organic ligands than metal hydroxides (I. E. R. L., 1979).

The pH value in a metal sulfide precipitation also affects metal species and, consequently, the chemical equilibrium in the system (Snoeyink and Jenkins, 1980; Francis et al., 1992; Chaberek and Martell, 1959; Li et al., 1959).

2.4. Concurrent removal of uranium and other heavy metals

In a waste stream that contains uranium, other heavy metals, and sulfate, the concurrent removal of uranium and other heavy metals by *D. desulfuricans* should be possible according to the theories of direct enzymatic uranium reduction and indirect enzymatic metal-sulfide precipitation. However, there will be some effects between these two mechanisms. In addition, if organic ligands are also present in the waste stream, the situation will be more complicated.

Lovley and Phillips (1992) studied a uranium-sulfate system. Sulfate was added to the 0.3-0.4 mM uranium solution to obtain a solution that contains 1.5-2.0 mM sulfate. They found that sulfate had no significant effect on U(VI) bioreduction, and U(VI) additions did not influence the rate of sulfate bioreduction; the two were reduced simultaneously. In contrast, Kucsmas (1994) found that sulfate presence could delay the uranium bioreduction rate when the sulfate concentration was higher than 10,000 mg/l (U(VI) concentration was 100 mg/l) or 5000 mg/l (U(VI) concentration was 10 mg/l). He also proposed that this delay might be caused by ionic strength inhibition or sodium inhibition.

Uranium has no toxicity to *D. desulfuricans*, but other heavy metals may have. Lovley and Phillips (1992) found that copper might cause uranium bioreduction rate decrease when copper concentration was higher than 40 μM. They also found that vanadium inhibited U(VI) reduction in a short-term (1.5 hours) but not over a long term (20 hours). Furthermore, they indicated that uranium reduction could not support

microorganism growth. Therefore, the presence of sulfate reduction could provide energy to support the growth of biomass.

In summary, research to date has focused on either direct enzymatic uranium reduction or indirect enzymatic metal removal by *D. desulfuricans*. In addition, most experiment solutions to date contained low concentrations of sulfate, or only one electron acceptor (uranium or sulfate). Unfortunately, most industrial wastewaters have much more complicated characteristics, yet may allow for the possibility of the two removal mechanisms to occur concurrently in the same system. Based on previous research above, the study presented here concentrated on employing the two mechanisms together in the more complicated industrial wastewater application.

CHAPTER III

EXPERIMENTAL METHODOLOGY

3.1. Experiment design

3.1.1. Synthetic wastewater experiment

Since the Y-12 Plant wastewater has complicated characteristics, the various pollutants in the wastewater could affect the treatment efficiency. Some of them could be major factors affecting *D. desulfuricans* activity. To investigate whether or not this biotreatment system is suitable for the Y-12 Plant wastewater, understanding the behavior of these pollutants during the treatment process was important. A synthetic wastewater experiment was conducted to limit the number of variables. Four major pollutants (uranium, sulfate, selected heavy metals, and chelating agents) were studied separately in different matrixes.

Uranium only matrixes These matrixes were used to investigate whether or not enzymatic uranium reduction can occur at low initial uranium concentration. Most researches about uranium reduction by *D. desulfuricans* have focused on high initial uranium concentration. However, this study focused on low concentration uranium reduction (4-6 mg/l) based on the Y-12 Plant wastewater. At low initial uranium concentration, the uranium bioreduction was evaluated, and then was compared with the reduction achieved at high initial concentration.

Uranium\sulfate matrixes These matrixes were used to investigate the potential competition between the two electron acceptors (uranium and sulfate) that were present in the same biotreatment system. Sulfate was added to the same low initial uranium concentration experiment previously described. The sulfate concentration levels tested were from 100 mg/l to 10,000 mg/l. These levels were chosen to cover the range of levels that could be present in the Y-12 Plant wastewater.

Uranium\sulfate\metal matrixes These matrixes were used to investigate the possibility of removal of other heavy metals. To understand the behavior of various metal ions in solution, zinc, nickel and copper were separately added to the synthetic solution containing 4-6 mg/l of uranium and 5,000 mg/l of sulfate. The Minteq Geochemical Assessment and Aquachem computer models were used to predict the possibility of sulfide precipitation on a theoretical basis. The concentrations of zinc, nickel, copper, uranium, and sulfate were chosen to simulate Y-12 Plant wastewater.

Uranium\sulfate\metal\organic ligand matrixes These matrixes were used to investigate the bioavailability of the uranium-ligand complex and the precipitation potential of the selected heavy metal-ligand complexes in the *D. desulfuricans* system. The Y-12 Plant wastewater contained various unknown organic chelating agents which may interfere with the metal removal. This study examined two common organic ligands, citrate and EDTA, as the representatives. Citrate and EDTA were separately added to the synthetic solutions containing uranium, zinc, nickel, and sulfate. The concentrations of uranium, heavy metals, and sulfate were chosen to simulate the Y-12 Plant wastewater. The concentrations of citrate and EDTA were chosen by a calculation

using the Minteq Geochemical Assessment computer model to make sure that all heavy metals were complexed by the organic ligand. Since there were no stability constants for uranium-citrate and uranium-EDTA in the Minteq computer model database, the quantity of uranium complexed by these organic ligands could not be predicted. However, this research concentrated only on the precipitation of zinc and nickel sulfides in organic ligand solutions.

Table 3 lists the main synthetic wastewater matrixes that were tested in this study. In addition, for uranium\sulfate\metal matrixes, four abiotic experiments were conducted to compare with biotreatment for evaluating the mechanism of metal removal in the biotreatment process. The abiotic experiment matrixes are listed in Table 4.

3.1.2. Y-12 Plant wastewater experiment

Experiment using actual wastewater from Y-12 Plant was conducted to compare results with the synthetic wastewater experiments. The wastewater came from the Y-12 Plant wastewater treatment facility, and lactate was also used as an electron donor in this experiment. This experiment was conducted to determine the feasibility of concurrent bioprecipitation in an actual DOE wastewater. The main characteristics of wastewater are listed in Table 5.

3.2. Bench scale reactor design

3.2.1. Biotreatment reactors

A 25 ml anaerobic pressure tube was used as the basic reactor unit. The pressure tube was sealed with a butyl rubber stopper and an aluminum cap to prevent the permeating of oxygen. In one reactor, the number of pressure tubes that were used

Table 3. Synthetic wastewater matrixes

Matrix	Uranium (mg/l)	Sulfate (mg/l)	Zinc (mg/l)	Nickel (mg/l)	Copper (mg/l)	Citrate (mg/l)	EDTA (mg/l)
Uranium only	4-10						
Uranium\ sulfate	4-10	500					
	4-10	1000					
	4-10	5000					
Uranium\ sulfate\ metal	4-10	5000	12-14				
	4-10	5000		12-14			
	4-10	5000			1		
	4-10	5000			3		
	4-10	5000			5		
	4-10	5000			10		
Uranium\ sulfate\ metal\ chelate	4-10	5000	12-14			3.78 g/l	
	4-10	5000	12-14				0.19
	4-10	5000		12-14		3.78 g/l	
	4-10	5000		12-14			0.19
	4-10	5000			1	3.78 g/l	
					1		0.19
	4-10					3.78g/l	
	4-10						0.19

Table 4. Abiotic experiment matrixes

Test number	Sulfide (mg/l)	Zinc (mg/l)	Nickel (mg/l)	Copper (mg/l)
1	33.33	12-14		
	83.33	12-14		
2	33.33		12-14	
	83.33		12-14	

Table 5. Y-12 Plant wastewater data

# Tank	pH	U(VI) (mg/l)	Zinc (mg/l)	Nickel (mg/l)	Copper (mg/l)	Sulfate (mg/l)	TOC (mg/l)
1	7.59	2.766	0.168	1.652	0.144	789.38	88
2	7.71	2.776	0.272	2.324	0.176	2123.76	46
3	7.92	8.501	2.54	3.304	0.592	1490.10	140
4	7.90	1.802	0.082	1.468	0.084	1170.87	189

depended on the sampling requirement. A sacrificial sampling technique was utilized to simulate the sampling in a single biotreatment reactor. For example, for a reactor that contained nine anaerobic pressure tubes, nine samples could be taken from this reactor. For each sample, one pressure tube was sacrificed.

3.2.2. Abiotic reactors

A 250 ml glass flask was used as an abiotic reactor. The solution in the reactor was mixed by the magnetic stirrer during the test period. The reactor was closed by a rubber stopper to prevent emitting hydrogen sulfide gas. A pH probe was placed through the top of reactor to monitor pH. The samples were taken from the side sampling point. The temperature for this reactor was maintained at room temperature.

3.3 Experimental materials and methods

The anaerobic techniques, *D. desulfuricans* growth media, and the inoculation method used in this study were developed by Lovley and Phillips (1992). The organism optimum growth phase (10^8/ml) for uranium removal utilized throughout the experiments was based upon the results of Ganesh (1993).

Source of the microorganisms The microorganism pure culture used in this study was *Desulfovibrio Desulfuricans*. The strain of *D. desulfuricans* (ATTCC 29577) was obtained from the American Type Culture Collection in Rockville, MD.

Making growth media Lovley and Phillips's (1992) growth media was used to grow *D. desulfuricans* in this study. It contained $NaHCO_3$ (2.5 g/l), NH_4Cl (1.5 g/l), NaH_2PO_4 (0.6 g/l), KCl (0.1 g/l), $MgSO_4$ (1.5 g/l), Na_2SO_4 (1.5 g/l), 60% lactic acid syrup (20 ml/l), yeast extract (2g/l), peptone (5g/l), mineral mix (10 ml/l), and vitamin

mix (10 ml/l). Since *D. desulfuricans* are anaerobic microorganisms, the growth media should be deoxygenated to ensure the viability of the microorganisms. An anaerobic technique based on Lovley and Phillips's study was used to create the anaerobic condition for degassing growth media. The growth media mixture was flushed for at least 10 minutes with a gas composed of 80 percent of hydrogen and 20 percent of carbon dioxide, then flushed for 5 minutes with a gas composed of 5 percent of carbon dioxide, 80 percent of nitrogen and 15 percent of hydrogen. The two gas mixtures were first passed through a column of hot reduced copper fillings to remove traces of oxygen before they were bubbled into the growth media. During the gas purging, the growth media were also maintained warm and mixed by a magnetic stirrer. Several 100 ml serum bottles were used to grow the microorganisms. The number of serum bottles used depended on the amount of microorganisms needed. Forty ml of degassed growth media were transferred into each of these serum bottles. The head space of each bottle was flushed with the CO_2-N_2-H_2 gas mixture for 1 minute to ensure anaerobic conditions, and then sterilized by an autoclave. Five ml of L-cysteine-HCL (5g/l) were added into each bottle by a syringe after the bottle was autoclaved, since it would precipitate at high temperature. L-cysteine-HCL here was added to help maintain the reduced condition in the growth media for sulfate reduction (Lovley and Phillips, 1992).

Culturing and harvesting cells The *D. desulfuricans* original pure culture was grown in a stock solution that was the same as the growth media. The cell concentration in the stock was around 10^8 cells/ml, which was analyzed by the "Acridine Orange Direct Count" procedure (Furcola et al. 1995). A one in ten dilution method was utilized to

dilute the microorganism stock solution to 10^4 cells/ml. Then five ml of the microorganism solution containing approximately 10^4 cells/ml was injected into each sterilized growth medium to get an initial concentration around 10^3 cell/ml in the serum bottle. After the microorganism injection, the bottles with growth media were put in an incubator at 35°C for 60 hours in order to obtain an optimum microorganism growth phase for uranium removal (Ganesh, 1993).

After 60 hours incubation, the clear growth media became turbid. The spectrophotometer (Beckman DU-70) showed that the absorbance of the media was around 0.6000. This indicated that the cells were in the optimum growth phase (10^8 cells/ml) for uranium reduction (Ganesh, 1993). The cells were transferred to centrifuge tubes and centrifuged at 11,000 rpm, 4°C for 25 minutes. The centrifuges that were used in this study were Beckman Model J2-21 and Eppendorf Centrifuge 5415C. The temperature during centrifuge was controlled at 4°C, since the microorganism metabolism is slow at this low temperature, which could reduce the oxygen toxicity for *D. desulfuricans*. After 25 minutes of centrifuging, the growth media suspension was decanted, leaving the cells at the bottom of the centrifuge tube. Then the cell precipitation was resuspended in an anaerobic bicarbonate buffer (2,500 mg/l sodium bicarbonate, 1.5 ml/l lactic acid), centrifuged and resuspended again to make sure that the growth media were washed out. The bicarbonate buffer solution was also degassed by H_2-CO_2 and CO_2-N_2-H_2 gases in the same way as that for the growth media. It was also sterilized by the autoclave and cooled to 4°C. The final cell suspension (0.5 ml in each centrifuge tube) was injected into various testing solutions. For a dead cell control

experiment, the cell suspension was placed in a boiling water bath for 15 minutes to heat-kill the cells, then this cell suspension was injected into the testing solution. The whole harvesting process should be carried out as quickly as possible to minimize the microorganism exposure to air.

Making test solutions The test solutions included all the synthetic wastewater matrixes that were listed in Table 3 and Table 4, as well as the real wastewater matrix. Sodium bicarbonate was used in the test solutions to provide a bicarbonate concentration. A 60% lactic acid syrup was used to provide lactate concentration. The atomic absorption standard solutions were used to provided zinc, nickel, and copper concentration. Sodium citrate and sodium EDTA were used to provided citrate and EDTA concentration. All test solutions (synthetic and real wastewater) were flushed with a gas mixture of H_2-CO_2 and H_2-CO_2-N_2 in the manner described above to obtain an anaerobic condition. Nine ml of test solution was transferred into a 25 ml pressure tube. The pressure tube containing the testing solution was autoclaved for sterilizing in the same way as described above for the growth media. A series of tubes served as the reactors. After injecting 0.5 ml of the microorganism suspension into the tubes, 0.5 ml of diluted uranium solution (around 80 mg/l) was injected into the tubes as well in order to obtain an initial uranium concentration around 4 to 6 mg/l. The diluted uranium solution came from a stock solution that was made from uranyl acetate. The stock uranium solution was also sealed with a butyl rubber stopper and an aluminum cap to prevent oxygen permeation. For those test solutions that contained zinc, nickel and copper, a sterilized filter system was used instead of an autoclave to prevent metal from

precipitating out of solution at high temperature. The filter system used was a 0.22 micron, nylon Corning Filtration System. For the chelating agent matrix, the microorganism injection was performed after one day of heavy metal addition to ensure that metal-ligand complex was formed (Shutteworth and Unz, 1991). For the abiotic test solution, sodium hydrogen sulfide was used to replace sodium sulfate to provide sulfide ions in the solution. Since there was no microorganism activity in the abiotic reactor, it did not require the deoxygenation process.

Taking samples The reactors with live cells, heat-killed cells, and no-cells were placed in a 30°C incubator. A five to eight ml sample was taken at days 0, 1, 2, 3, and 4, or hours 0, 1, 2, 4, 8, 12, 24, 36, 48, 72, and 96 for the live cell matrixes. For the heat-killed cell matrixes, the sampling times were days 0, 2, and 4, or hours 0 and 96. For the no-cell matrixes, the sampling time were days 0 and 4. The samples were filtered through the 0.2 μm (Gelman Sciences, Sterile Acrodisc) syringe filters to separate the cells and precipitate from the samples, and placed in a 4°C cool room to terminate further microorganism activity before they were analyzed. For the abiotic metal sulfide precipitation experiment, the samples were taken at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, and 150 minutes. The samples were also filtered by the 0.2 μm syringe filters to separate the heavy metal precipitation from the samples.

3.4. Analytical methods

Uranium concentration was measured by a Kinetic Phosphorescence Analyzer (KPA-11, Chemchek Instruments, Richland, WA). The instrument uses a pulsed nitrogen

dye laser and a complexing agent (URAPLEX) to measure hexavalent uranium (UO_2^{+2}) in the solution. The URAPLEX was used to decrease the interference of other compounds present in the solution. The number of laser pulses used for measurement was 1,000 pulses. The instrument was calibrated with an uranyl nitrate standard solution. An instrumental background and a uranium reference solution were used to correct the variables in order to improve the accuracy and precision of the results. The sample volume used for measurement was 1.0 ml of sample plus 1.5 ml of URAPLEX.

The uranium sample dilution factors that were used in this study were 4, and 80, depending on the test solutions. The tetravalent uranium (UO_2) is not stable if it does not precipitate from solution. Therefore, when a uranium sample is exposed to air, the tetravalent uranium could be oxidized back to its hexavalent state by oxygen (Anderson, 1984). To prevent U(IV) from oxidizing to U(VI) again, uranium samples were analyzed immediately after they were taken. Also the distilled water used to dilute uranium samples was deoxygenated. The remaining samples were kept in a 4°C cool room to preserve for any other analyses necessary. In this research, four types of uranium were measured: U(VI)-unfitered, U(VI)-filtrate (aqueous U(VI) concentration), total uranium-unfiltered, and total uranium-filtrate. To measure U(VI)-unfiltered, the samples were collected from the pressure tubes and appropriately diluted using deoxygenated distilled water. Then they were directly analyzed for the U(VI) concentration by the KPA instrument. The difference of measuring U(VI)-filtrate from measuring U(VI)-unfiltered was to pass the collected samples through a 0.2 μm filter before the dilution. To measure total uranium-unfiltered, the collected samples were oxidized by 2 N nitric acid solution

first, then were directly analyzed for the U(VI) concentration by the KPA instrument. The difference of measuring total uranium-filtrate from measuring total uranium-unfiltered was to pass the collected samples through a 0.2 μm filter before oxidized by 2 N nitric acid. The difference between the total uranium-unfiltered and U(VI)-filtrate was counted for U(IV) formation.

Zinc, nickel and copper were analyzed by a flame ionization atomic absorption (AA) spectrophotometer. The air acetylene flame was used in the measurement. The absorption wavelength used for zinc, nickel, and copper were 213.9, 232.0, and 324.7 nm, respectively. The dilution factors for zinc, nickel, and copper samples are 20, 4, and 4, and the detection limits were 0.012, 0.12 and 0.024 mg/l, respectively. The atomic absorption standard solutions were used to calibrate the instrument. For the abiotic metal sulfide precipitation experiments, the samples were first filtered by a 0.2 μm filter to separate the metal sulfide from the solutions. Since chemical reaction was still progressing in the sample, the sample filtrate was treated by 12N nitric acid to dissolve metal sulfide produced. Then it was measured by AA for metal concentration.

Sulfate concentration was measured by a Perkin Elmer Series 410 High Performance Liquid Chromatography with an Ion-120 ion exchange column (Interaction Chromatography Inc.). A 4.0 mM salicylate buffer was used as a column eluent with a flow rate of 1.3 ml/min. A UV detector was used at a wavelength of 254 nM. Sodium sulfate solution was used as a standard solution. The sample dilution factors were 0, 10, 50, and 100, depending on sulfate concentration in the sample. pH was measured by a Corning 340 pH meter.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. U(VI) reduction at low initial concentration

Most research for uranium enzymatic reduction has concentrated on high initial concentration. In order to investigate uranium reduction potential and efficiency at a low initial concentration such as that of the Y-12 Plant wastewater, a simplified synthetic wastewater containing 2,500 mg/l of bicarbonate, 10 mM of lactate (as electron donor), and 4-6 mg/l of uranium was used as a test solution. After inoculating *D. desulfuricans* into the test solution that contained a low concentration of uranium, samples were taken at 0, 1, 2, 4, 8, 12, 24, and 36 hours. The dead cell (heat killed) reactors were used as the control to compare with the viable cell reactors. Samples were taken from the dead cell reactors at 0 and 36 hours.

The results are presented in Figures 6-8. Figure 6 indicates that *D. desulfuricans* can efficiently remove U(VI) at low initial concentrations (4-6 mg/l). This result is similar to Lovley and Phillips's result (1992), which was at a high initial uranium concentration. A black precipitant was formed on the bottom of viable cell tubes after 24 hours. According to the X-ray diffraction analysis in Lovley and Phillips's research (1992), this black precipitation was made up of the U(IV)-containing mineral uraninite (UO_2). Transmission electron microscopy also indicated that this uranium precipitation was extracellular (Lovley and Phillips, 1992). Figure 6 shows that rapid U(VI) removal

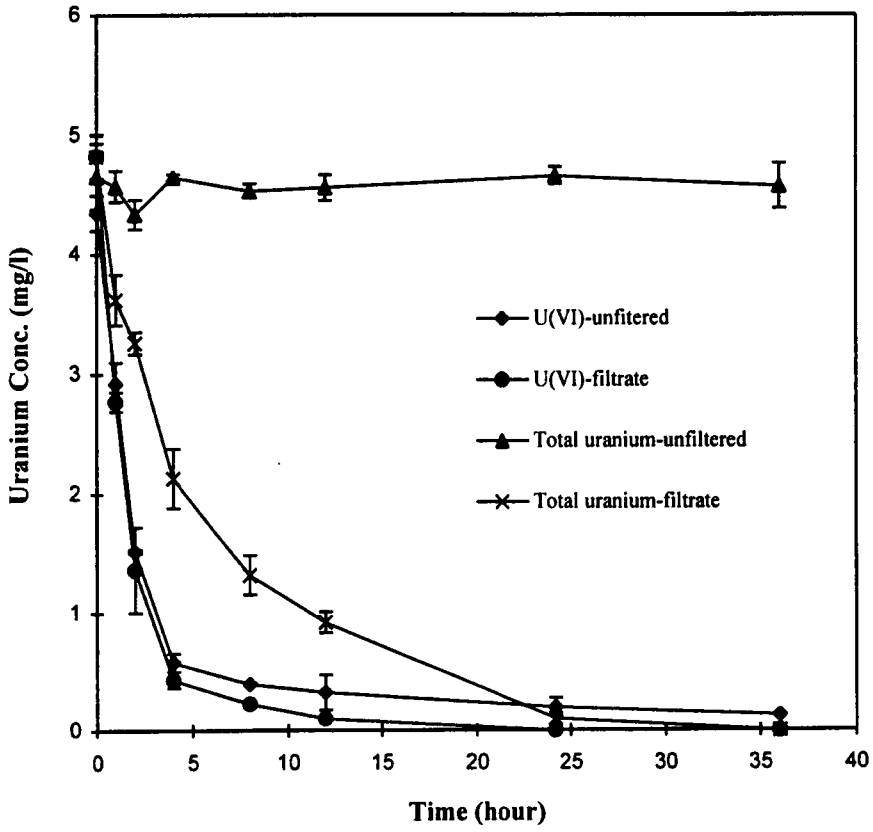

Figure 6. U(VI) reduction and uranium precipitation in low initial concentration by *D. desulfuricans* in a bicarbonate solution as a function of time. The electron donor was lactate.

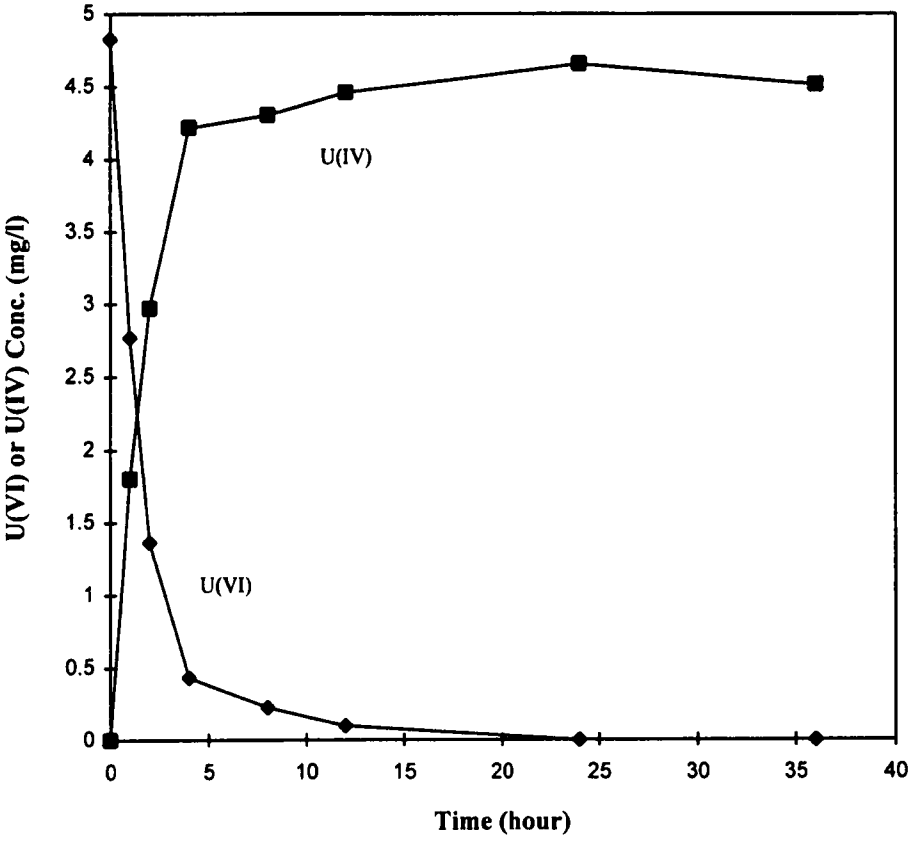

Figure 7. Reduction of U(VI) to U(IV) by *D.desulfuricans* as a function of time. Lactate was used as the electron donor.

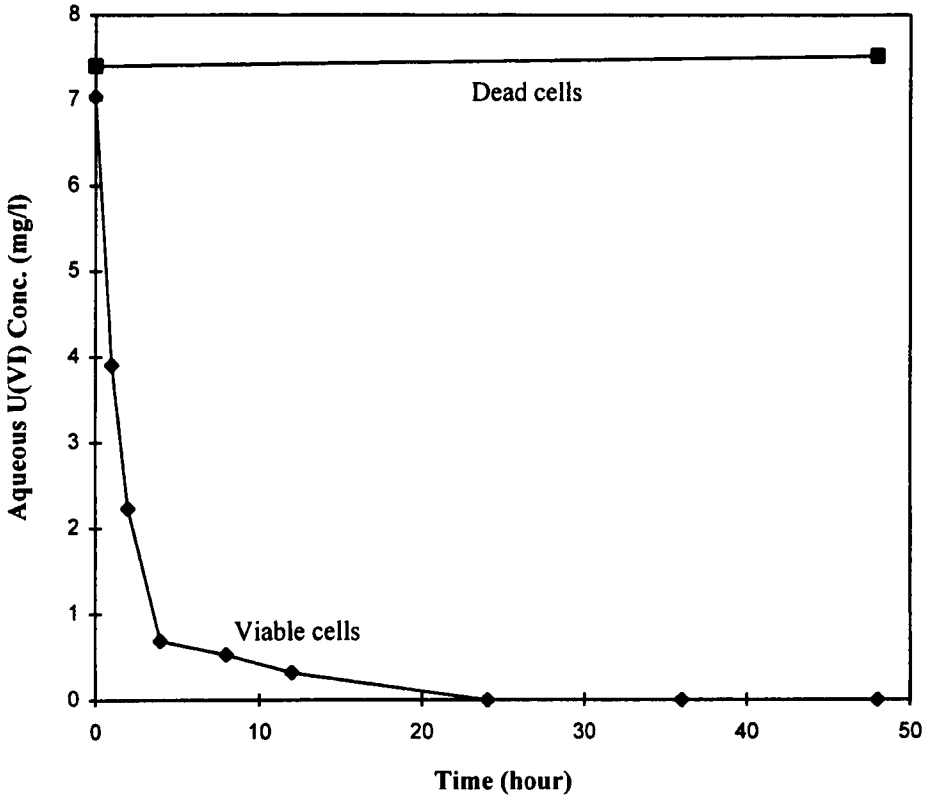

Figure 8. Removal of U(VI) from bicarbonate solution by *D. desulfuricans* as a function of time. Heat killed cells were used as a control.

occurred in the unfiltered samples as well, while the total uranium remained constant. That indicates the removal mechanism was bioreduction as opposed to biosorption. Before the first 24 hours, there was a certain amount of U(IV) that could not be separated from the sample by filtration, indicating that the initial U(IV) produced was soluble or colloidal. This also demonstrated how important an immediate sample analysis was, since soluble U(IV) was easily oxidized back to soluble U(VI) when it was exposed to air. However, nearly all the U(VI) in the solution was reduced to insoluble U(IV) after 24 hours. Figure 6 also shows that a small amount of U(VI) could be still present in the unfiltered sample until 36 hours. The explanation for this phenomenon may be that the precipitation of U(IV) caused co-precipitation of a small amount of U(VI), which was then no longer available for reduction (Lovley and Phillips, 1992). Figure 7 shows that U(VI) decreased while corresponding U(IV) increased during the test period. In this figure, the U(VI) concentration was determined by immediately analyzing the filtered samples. U(IV) concentration was determined by subtracting the U(VI) concentration in the filtered samples from total U(VI) concentration in unfiltered samples. There was almost no U(VI) removal in the dead cell control samples (Figure 8). This result also suggests that microbial reduction was the main cause of U(VI) removal from the solution rather than biosorption.

In addition, a high concentration of bicarbonate (2,500 mg/l), as an inorganic ligand, was in the synthetic wastewater. According to a Minteq computer program calculation, the U(VI) would be in the form of uranyl-carbonate complexes. The results of this study indicated that *D. desulfuricans* can rapidly convert a low concentration of

U(VI) (4-6 mg/l) to U(IV) in a carbonate complex situation, which was the same result as in high concentrations of U(VI) (Lovley and Phillips, 1992). Therefore, for an uranium contaminated waste stream that contains a carbonate ligand, microbial uranium reduction has the potential to precipitate uranium from the solution.

Comparing uranium removal in high and low initial concentrations, the uranium removal process is slower at low initial concentration than at the high initial concentration used by Lovley and Phillips (1992). Figure 9 shows that uranium bioreduction in low initial concentration is approximately a first order reaction, that is, the reduction rate is directly proportional to the uranium concentration. Therefore, the reduction rate can be described by the following equation:

$$\frac{d[C]}{dT} = -K[C]$$

where:

dC/dT = rate of change in U(VI) concentration with time.

C = U(VI) concentration at any time, t.

K = reduction rate constant, from Figure 9, K=0.3113 hr^{-1} (in this study condition).

Based on this equation, the uranium reduction rate will decrease with a decrease in uranium concentration in the solution, so the uranium reduction rate should be lower at a

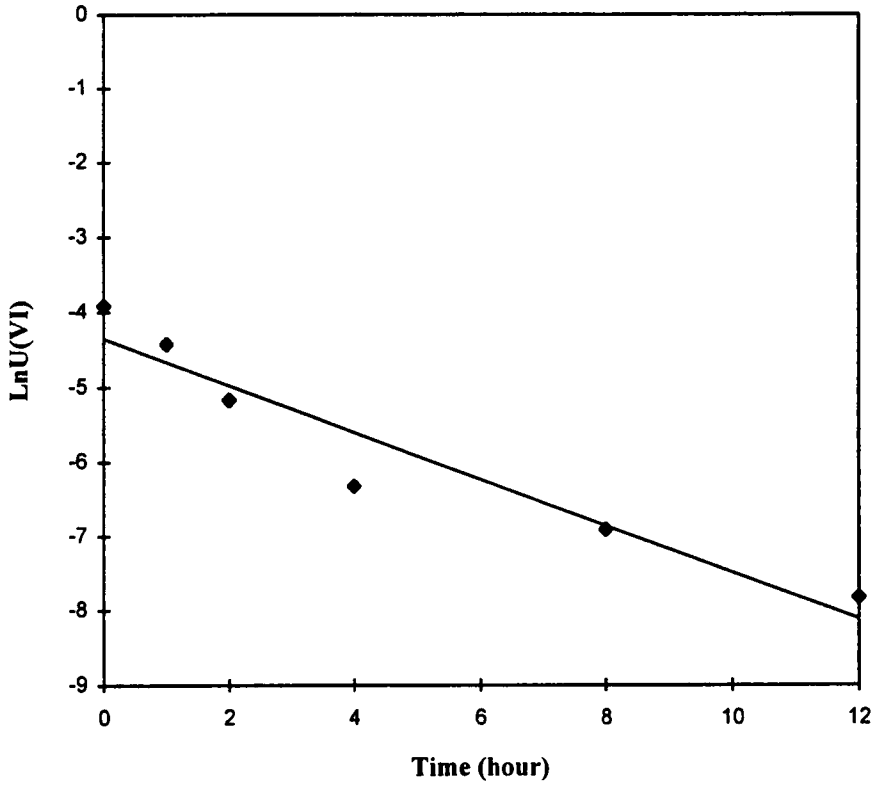

Figure 9. LnU(VI) as a function of time. U(VI) data were presented in Figure 6.

low initial concentration than at a high initial concentration. However, according to the rate constant determinations, as shown in Appendix II, the rate constant of uranium reduction in low concentration (K=0.3113 hr^{-1}) is different from that in high concentration (K=1.1822 hr^{-1}) described by Lovley and Phillips (1992). This result is consistent with that of the experiment for low concentration uranium reduction conducted by Lovley and Phillips (1992). Their low concentration uranium reduction curve has a K value of 0.5126. The difference of value of K between low concentration and high concentration uranium reduction could be caused by the low uranium concentration. A 4-6 mg/l of uranium may not be high enough to induce microorganisms to metabolize at their maximum enzymatic activity.

4.2. Uranium reduction in sulfate containing solution

The above experiment of low initial uranium concentration demonstrated that *D. desulfuricans* could efficiently reduce soluble U(VI) to insoluble U(IV) by enzymatic reaction. Since the Y-12 Plant wastewater contains high concentration of sulfate (around 5,000 mg/l), which is also an electron acceptor for *D. desulfuricans*, sulfate could compete with uranium for electrons. Although Lovley and Phillips (1992) showed that in their experiment there was no competition between uranium and sulfate and both can be reduced simultaneously, their results were based on high uranium concentration (100 mg/l) and low sulfate concentration (200 mg/l). Kucsmas (1994) observed that the competition between uranium and sulfate was different in high and low uranium concentration. In one of his experiments with low uranium concentration (10 mg/l), he observed the ultimate amount of uranium removed decreased as the sulfate concentration increased. The sulfate

concentrations he tested were 1,000, 5,000, and 10,000 mg/l. The current research followed up on the work of Kucsmas (1994) and investigated the sulfate effect in more detail, especially the effect of high sulfate concentration on the reduction of uranium at low concentration.

There are three other factors, besides sulfate, that may also affect uranium reduction in the sulfate solution:

- Ionic strength: the higher the sulfate concentration, the higher the ionic strength in the solution. A high ionic strength could affect the osmosis across a bacteria membrane, and consequently affect the organism activity. Therefore, high ionic strength may cause a decline in uranium reduction.

- Sodium concentration: the higher the sulfate concentration, the higher the sodium concentration can be in the solution. Sodium concentration could also affect osmosis, and inhibit the bioactivity of *D. desulfuricans*.

- Electron donor limitation: the bioreduction of both uranium and sulfate requires enough electron donor (lactate) to complete the bioenergetic process. If the sulfate (electron acceptor) concentration is high, the lactate may become a limiting factor. Therefore, electron donor limitation could cause uranium reduction decline.

To investigate the effect of high concentration sulfate on low concentration uranium reduction, four experiments, experiment A, B, C, and D, were conducted.

Experiment A. The purpose of experiment A was to investigate whether the level of sulfate concentration could affect uranium removal by *D. desulfuricans*. Parallel reactors were used in this experiment. The synthetic wastewater for this experiment contained 2,500

mg/l of bicarbonate, 10 mM of lactate (serving as an electron donor), and 4 to 6 mg/l of uranium. The test solutions also contained sulfate in concentrations of 0, 500, 1,000, 5,000, and 10,000 mg/l. Two dead cell (heat-killed) reactors, which contained 0 and 10,000 mg/l of initial sulfate concentrations, respectively, were used to compare with viable cell reactors. Samples were taken at 0, 1, 2, 4, 8, 12, 24, 36, and 48 hours. When the uranium/sulfate matrixes were sampled, a strong hydrogen sulfide odor was noticed, indicating that *D. desulfuricans* was reducing sulfate to sulfide. The sulfate levels during the experiment also indicate that the sulfate reduction occurred in all sulfate containing matrixes (Table 6). The results for uranium removal in different sulfate concentrations are shown in Figure 10 and suggest that when sulfate concentrations increased from 0 to 1,000 mg/l, the extent of uranium removal gradually decreased. When the sulfate concentration was increased to 5,000 mg/l, a significant drop on the ultimate amount of uranium removal was observed. When the sulfate concentration was increased from 5,000 to 10,000 mg/l, the decrease on the ultimate amount of uranium removal again became insignificant. On the other hand, as initial uranium to sulfate ratio decreased, the ultimate uranium removal efficiency decreased (Table 7). This decrease suggests possible competition between uranium and sulfate. At low uranium to sulfate ratios, sulfate may be the dominant electron acceptor, even though uranium is preferred thermodynamically in the following chemical equations.

$$SO_4^{-2} + 2\ C_3H_6O_3 \rightarrow 2\ C_2H_4O_2 + 2CO_2 + S^{-2} + H_2O \qquad (1)$$

$$\Delta G^0 = -236\ kj$$

$$UO_2^{+2} + C_3H_6O_3 \rightarrow UO_2 + C_2H_4O_2 + 2H^+ + CO_2 \qquad (2)$$

Table 6. Sulfate reduction in defferent initial concentrations

Time (hour)	Sulfate Conc. (mg/l)			
0	447.85	935.96	5032.21	9822.13
1	462.62	880.62	4963.65	9822.24
2	353.70	805.98	4658.49	9116.47
4	325.70	793.52	4674.78	9394.28
8	155.34	588.21	4564.13	8735.60
12	82.98	442.30	4499.72	8721.53
24	44.11	444.62	4196.89	8949.73
36	0.00	371.19	4063.39	7755.04
48	25.59	385.94	4062.34	8410.11

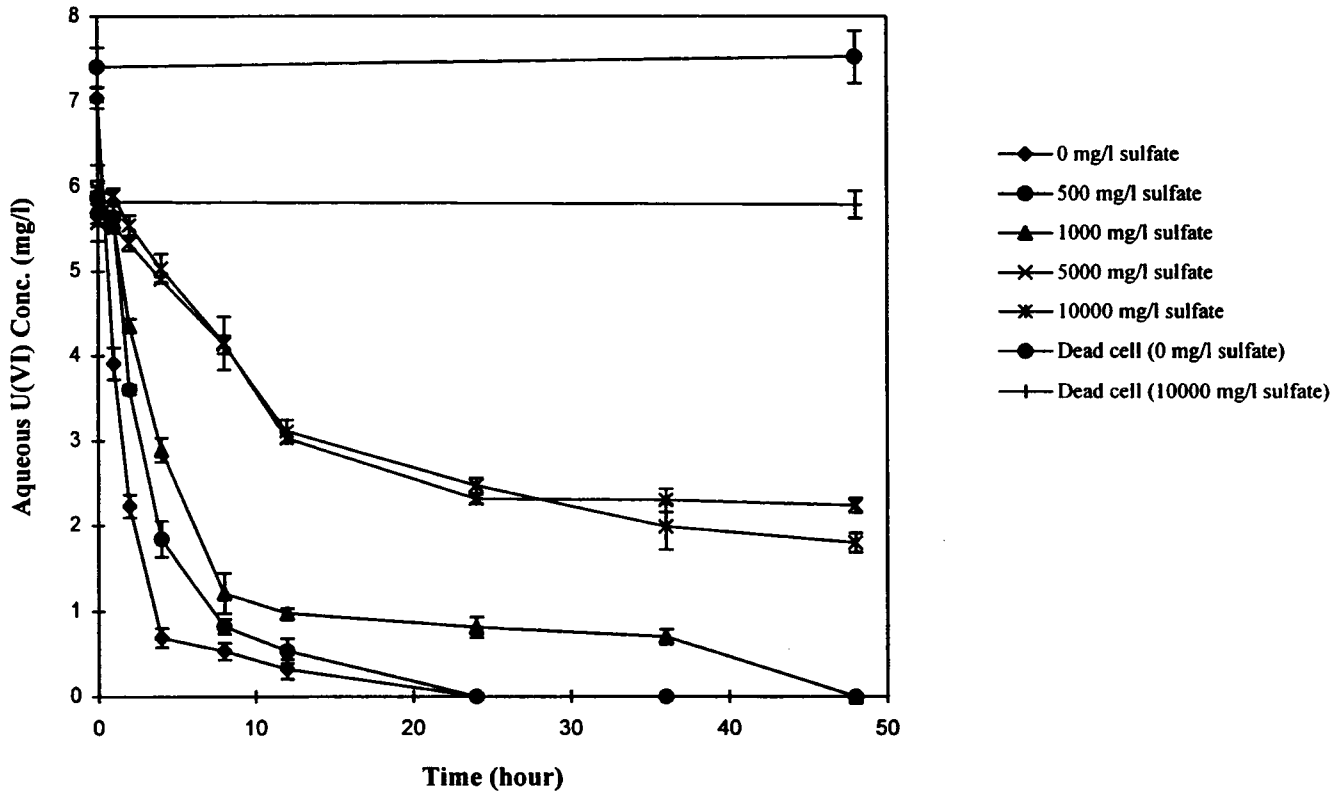

Figure 10. U(VI) removal from sulfate solutions by *D. desulfuricans* as a function of time. Lactate was used as the electron donor.

Table 7. U/SO_4 ratio effect on uranium removal efficiency

Initial Concentration (mg/l)			U Removal Eff. (%)	
Uranium	Sulfate	U/SO_4 (mol/mol)	24 hour	48 hour
5.875	447.85	0.0054	100	100
5.760	935.96	0.0025	85.95	100
5.576	5032.21	0.0004	55.72	67.75
5.968	9822.13	0.0002	61.24	62.52

$\Delta G^0 = -335$ kj

The results of the dead cell reactors showed almost no change in uranium and sulfate levels during the test period, which indicates that enzymatic reduction caused uranium and sulfate removal.

Experiment B. The purpose of experiment B was to evaluate the effect of ionic strength on uranium removal in the test solution. The basic idea for this experiment was to observe the difference in uranium removal by keeping ionic strength the same in the solution and changing the sulfate concentration. Experiment B was conducted in two reactors. Both of them contained 2,500 g/l of bicarbonate, 10 mM of lactate, and 4 to 6 mg/l of uranium. The two reactors had same ionic strength but different sulfate concentrations. One of them contained 10,000 mg/l of sulfate and the other contained 500 mg/l of sulfate. For the 500 mg/l sulfate matrix, sodium nitrate (21.94 g/l) was added to maintain the same ionic strength (around 0.29 M) as that of the 10,000 mg/l sulfate matrix. The amount of sodium nitrate added was calculated by the Minteq computer program. Since nitrate is reduced by *D. desulfuricans* after 2 to 4 days inoculation (Dalsgaard and Bak, 1994), the ionic strength of the test solution would not change during this experiment period of 2 days. The results graphed in Figure 11 indicate that when the ionic strength was the same, the amount of uranium removal did not show a significant difference between the 500 and 10,000 mg/l of sulfate solutions, especially within one day. The rate constants of uranium removal (Appendix II) also indicate that the two curves do not have significant difference. Since there was a slight difference on the uranium removal between these two conditions after one day, sulfate/uranium competition should not be totally ruled

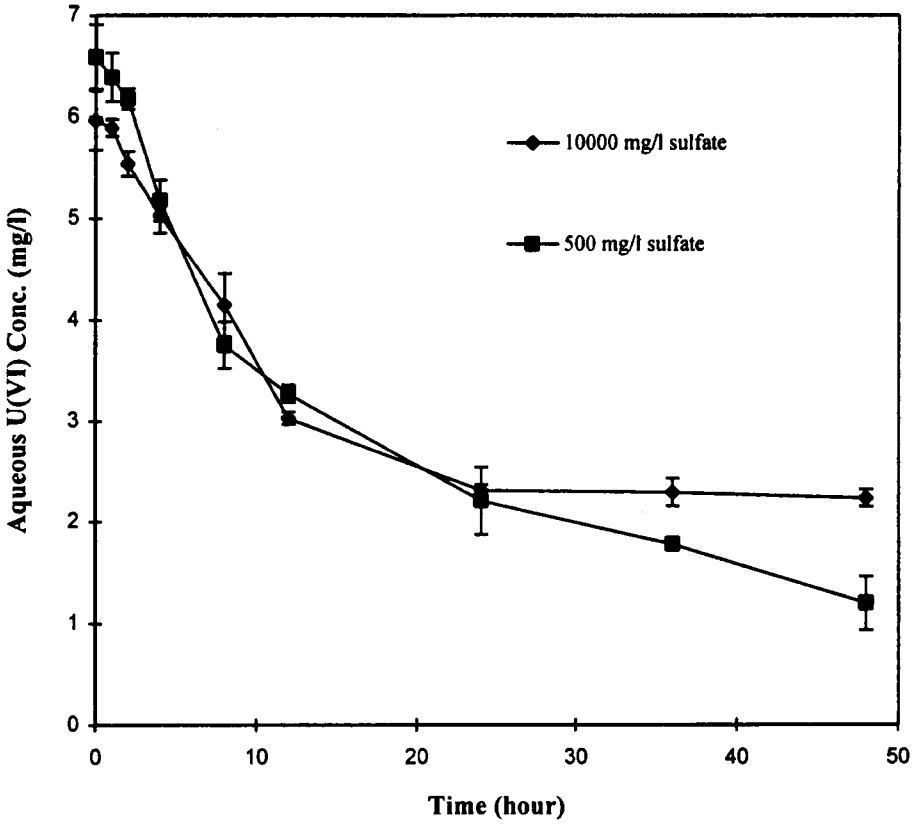

Figure 11. U(VI) removal from the solutions containing same ionic strength (0.29) and different sulfate concentrations as a function of time. The electron donor was lactate.

out. However, the ionic strength in the solution appears to be the main cause of reduced uranium removal. In addition, comparing two 500 mg/l sulfate reactors in experiment A and experiment B, they contained same sulfate concentration but different ionic strength. The reactor containing high ionic strength had a low rate constant of uranium removal (Appendix II), which indicates that the ionic strength in the solution appears to be the main cause of reduced uranium removal.

Experiment C. The purpose of experiment C was to determine if sodium concentration is an affecting factor of the uranium reduction. Two reactors were used for this experiment. Each of them contained 2,500 mg/l of bicarbonate, 10 mM of lactate, and 4 to 6 mg/l of uranium. The two reactors in experiment C had same sodium concentrations (0.13 M) but different sulfate concentrations and different ionic strength. One reactor contained sodium sulfate (5,000 mg/L as SO_4^{-2}), and the other contained sodium nitrate (6,448 mg/L as NO_3^{-1}). The results are graphed in Figure 12. The significant difference in the uranium removal between these two curves suggests that sodium concentration was not the main reason for the reduced uranium removal. Therefore, sodium concentration is not an important affecting factor of the uranium reduction under this experimental condition. The difference in the uranium removal may be caused by the variation in ionic strength and sulfate concentration. However, comparing two reactors in this experiment, sulfate concentration changed from 5000 mg/l to 0 mg/l, and ionic strength only changed from 0.17 to 0.13. It appears that the difference in the uranium removal may be mainly caused by sulfate concentration.

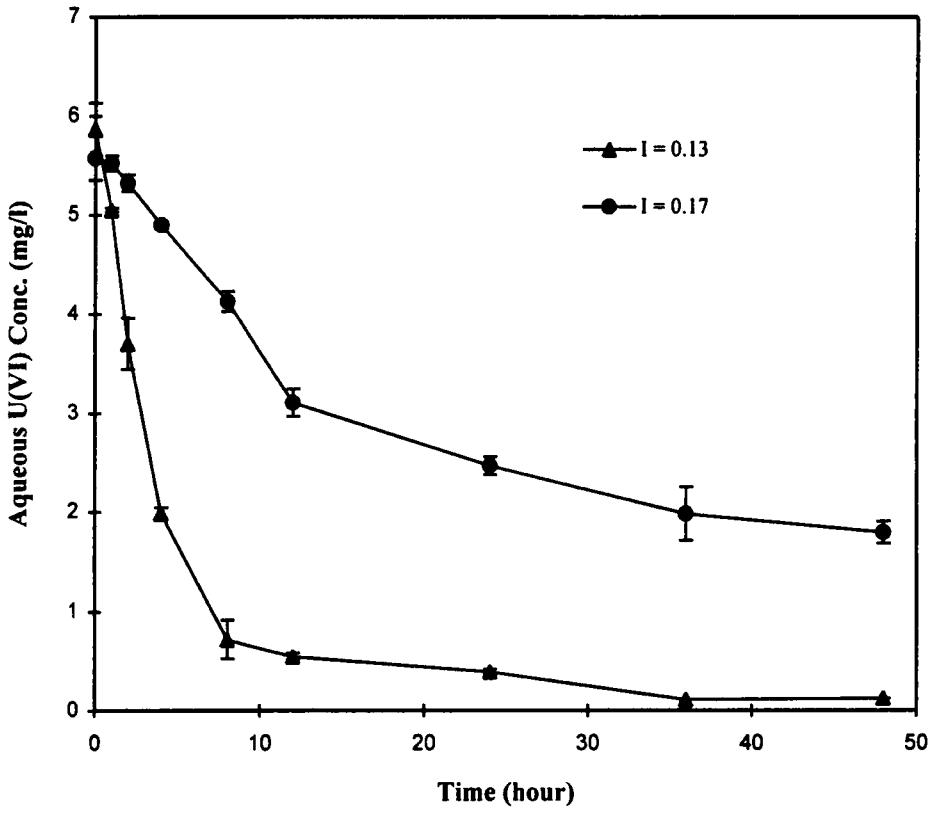

Figure 12. U(VI) removal from the solutions containing same sodium concentration (0.13 M) and different ionic strength as a function of time. The electron donor was lactate.

Experiment D. Experiment D was conducted to investigate the effect of electron donor limitation on the uranium and sulfate reduction rates. An electron donor (lactate) limitation could be another factor that may affect the uranium removal rate in the solution. The possible reactions for uranium reduction and sulfate reduction with lactate are shown in the equations 1 and 2 discussed before. From stoichiometric calculations, 10 mM of lactate can give enough electrons for 4 mg/l of U(VI) and 1,000 mg/l of SO_4^{-2} reduction, but not enough electrons for 4 mg/l of U(VI) and 5,000 mg/l of SO_4^{-2} reduction. Therefore, the declining of uranium removal occurring above 5,000 mg/l of SO_4^{-2} may be caused by a shortage of lactate.

Four reactors were used in this experiment. The reactors contained 2,500 mg/l of bicarbonate and four different levels of sulfate concentrations (0, 500, 1,000, and 5,000 mg/l). The difference between experiments D and A was that the lactate level was decreased from 10 mM in experiment A to 2 mM in experiment D, which made it more possible to be electron donor limited. From stoichiometry, 2 mM of lactate can not even provide enough electrons for uranium removal in a solution of 4 mg/l of U(VI) and 500 mg/l of SO_4^{-2}. The results for experiment D are plotted in Figures 13 and 14. Figure 13 shows that even when the electron donor was limited, uranium removal appeared to have a similar trend compared to experiment A (Figure 10). A statistical analysis using the Cox and Stuart Test (Appendix II) also indicated there was no significant decrease on uranium removal in 2 mM of lactate when compared to that in 10 mM of lactate. Figure 14, however, shows that the ultimate amount of sulfate removal changed significantly between 10 mM of lactate and 2 mM of lactate. In the 2 mM lactate situation, the sulfate appeared

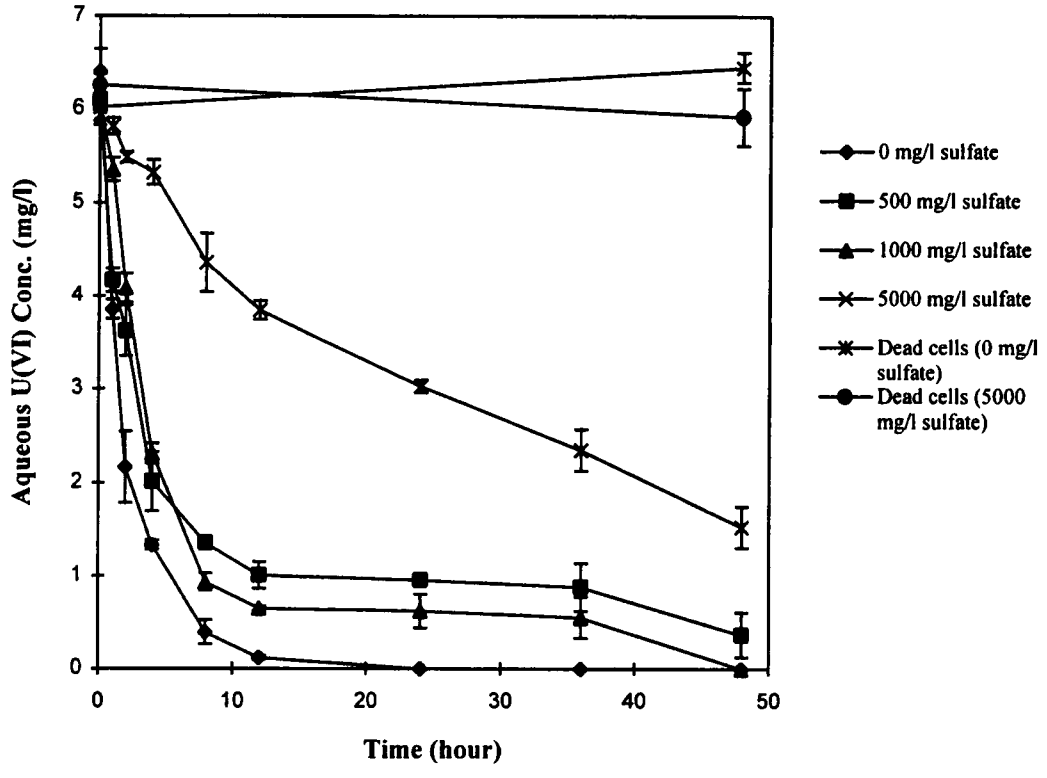

Figure 13. U(VI) removal from sulfate solutions as a function of time. 2mM lactate was used as the electron donor.

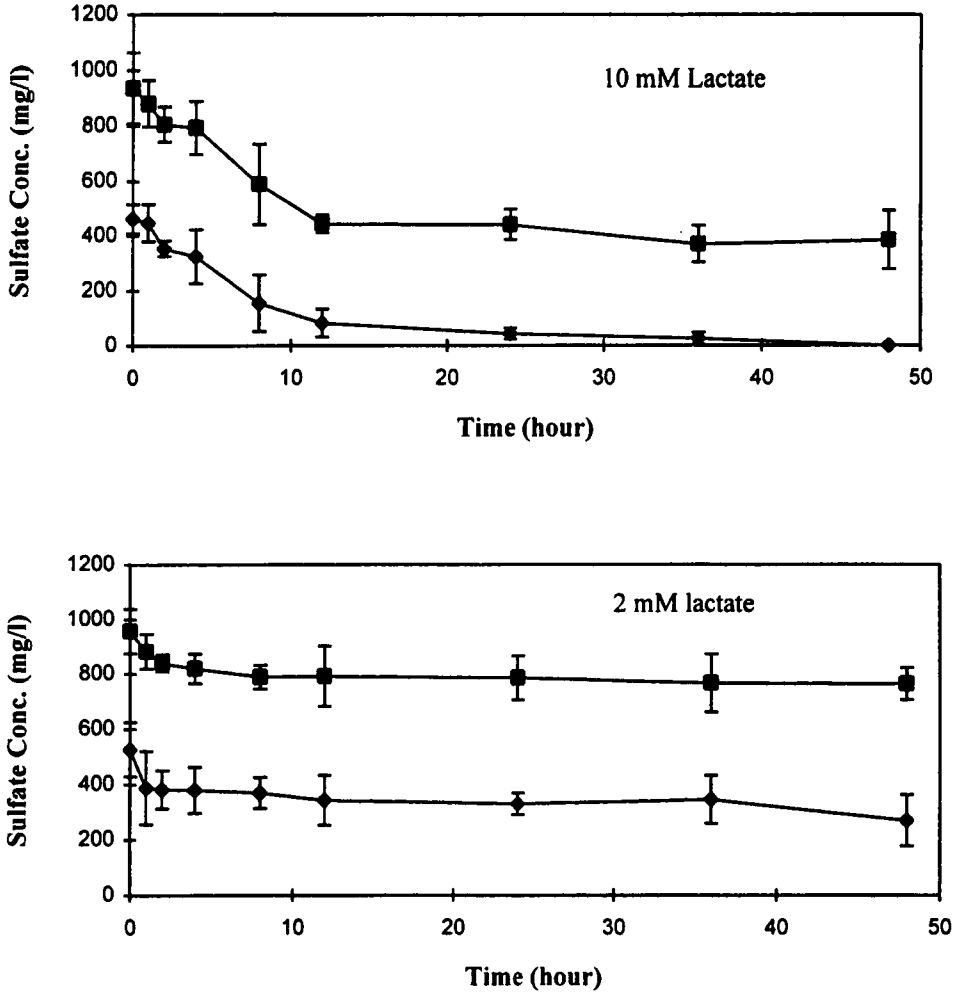

Figure 14. Sulfate removal from different initial concentration solutions by *D. desulfuricans* as a function of time. The electron donor was lactate.

more electron limited for their enzymatic reduction than in 10 mM lactate situation. These results can be described by chemical equilibrium thermodynamics. Comparing free energy (ΔG^0) released from the chemical equations, the uranium reduction is more thermodynamically favorable than the sulfate reduction. When enough electron donor was provided in the solution, no obvious competition appeared between uranium reduction and sulfate reduction (Lovley and Phillips, 1992). When the electron donor was limited, the sulfate removal decreased whereas the amount of uranium removal remained apparently constant. Thus, in the condition of experiment A (10 mM of lactate), when sulfate concentration was higher than 5,000 mg/l, the electron donor limitation might affect both sulfate and uranium reduction, however, it was probably not the main cause of reduced uranium removal.

Summarizing experiment A, B, C, and D, when the solution contained 4-6 mg/l of uranium, along with sulfate concentration was increased from 0 to 10,000 mg/l, the effectiveness of *D. desulfuricans* for uranium removal could decrease. This decrease could be caused by a combined effect of high ionic strength, electron acceptor competition, and electron donor limitation. According to the rate constants of uranium removal in Appendix II, in a low ionic strength condition (experiment A and C), the effect of sulfate concentration appears to be more important than that of ionic strength. In contrast, in a high ionic strength condition (experiment A and experiment B), the effect of ionic strength appears to be more important than that of sulfate concentration.

4.3. Concurrent removal of other heavy metals with reduction of uranium

The other heavy metals such as zinc, nickel, and copper presented in the Y-12 Plant wastewater have a potential to be concurrently removed with uranium by metal sulfide precipitation, since *D. desulfuricans* can produce sulfide ions from sulfate in the wastewater (Ehrlich and Brierley, 1990). For this mechanism of indirect enzymatic metal removal, several factors may affect the process. To investigate this metal removal potential, four experiments, experiment E, F, G, and H, were conducted to determine the possible reaction mechanism and the effects of the metal toxicity.

Experiment E. The purpose of Experiment E was to investigate the potential for concurrent heavy metal removal with uranium reduction. According to the result of Experiment A, *D. desulfuricans* can reduce approximately 500 mg/l of sulfate to sulfide per day in a uranium/sulfate solution under the test conditions. The concentration of zinc (Zn^{+2}), nickel (Ni^{+2}), and copper (Cu^{+2}) in the test solution are 12, 12, and 10 mg/l, respectively. By calculation the amounts of sulfate required to produce sulfide then to precipitate these heavy metals are 17.6 mg/l for zinc, 19.6 mg/l for nickel and 15.1 mg/l for copper. Therefore, the sulfate microbial reduction process may likely provide enough sulfide ion for concurrent heavy metal precipitation.

Based on the uranium/sulfate matrix in experiment A, three reactors were used in experiment E. Solutions of 12 to 14 mg/l of zinc, 12 to 14 mg/l of nickel, and 8 to 10 mg/l of copper each were added to the synthetic wastewater containing 2,500 mg/l of bicarbonate, 10 mM of lactate (serving as the electron donor), and 5,000 mg/l of sulfate. The results are plotted in Figures 15, 16, and 17. Figures 15 and 16 indicate that the

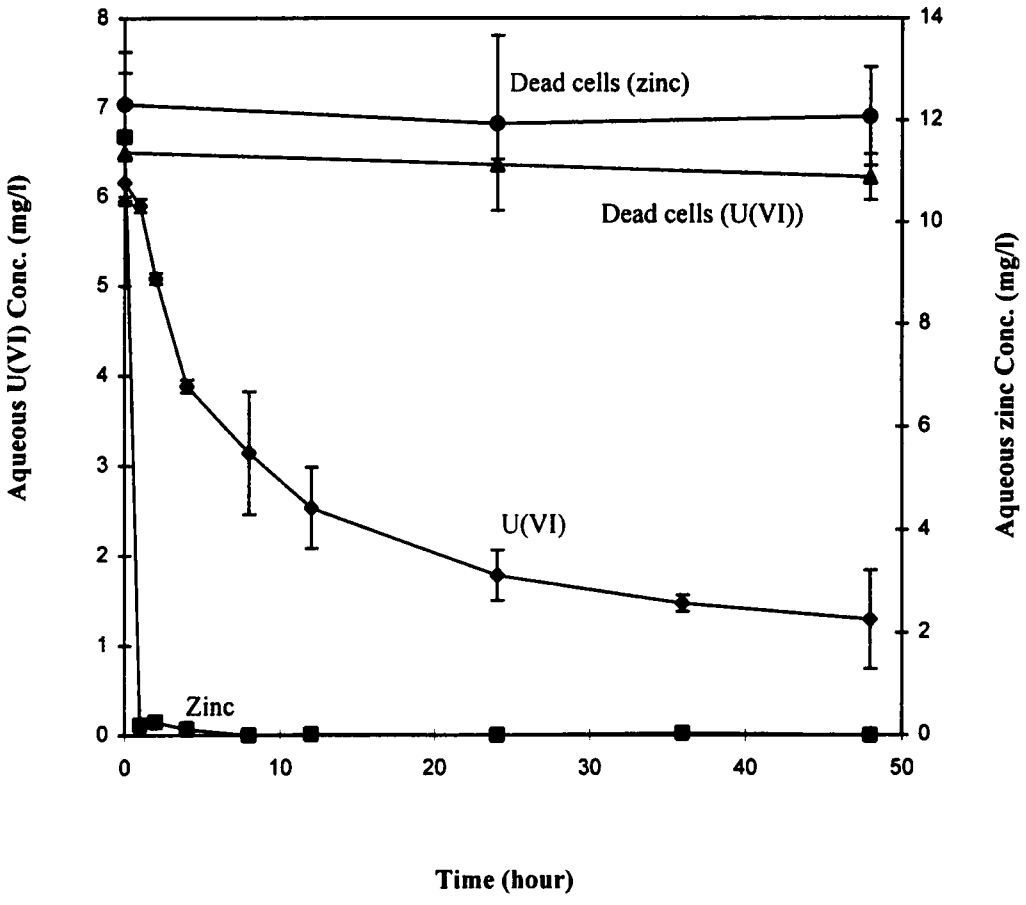

Figure 15. Removal of U(VI) and zinc from the solution containing 5,000 mg/l sulfate after inoculation with *D. desulfuricans*, as a function of time. The electron donor was lactate.

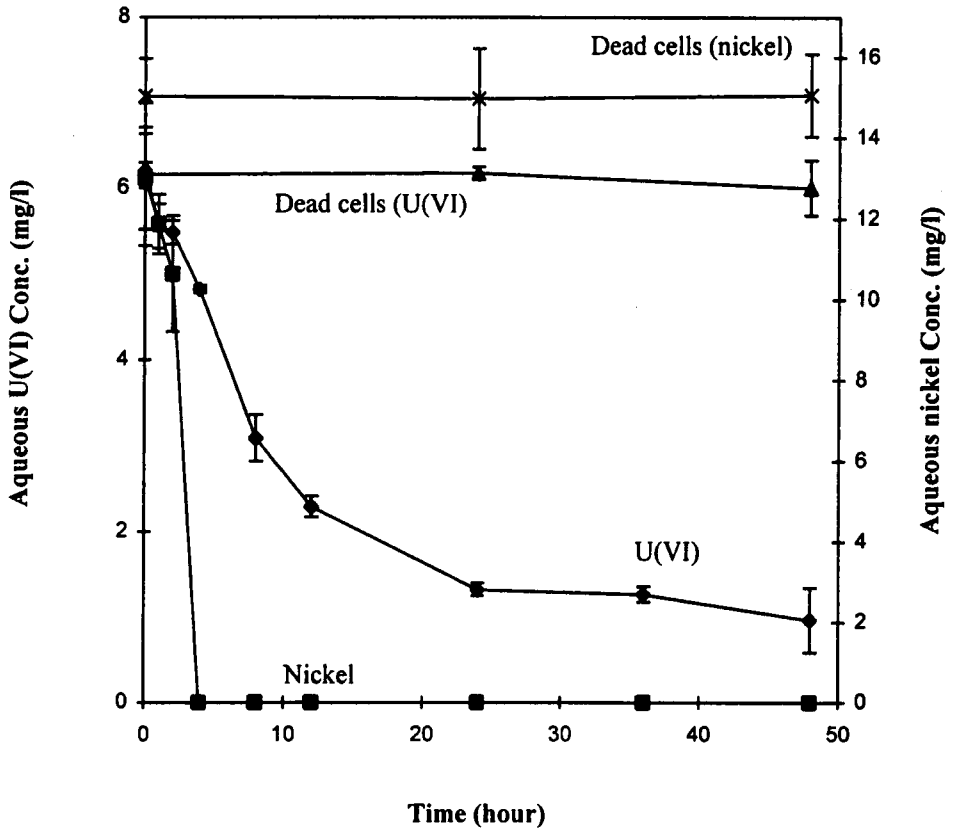

Figure 16. Removal of U(VI) and nickel from the solution containing 5,000 mg/l sulfate after inoculation with *D. desulfuricans,* as a function of time. The electron donor was lactate.

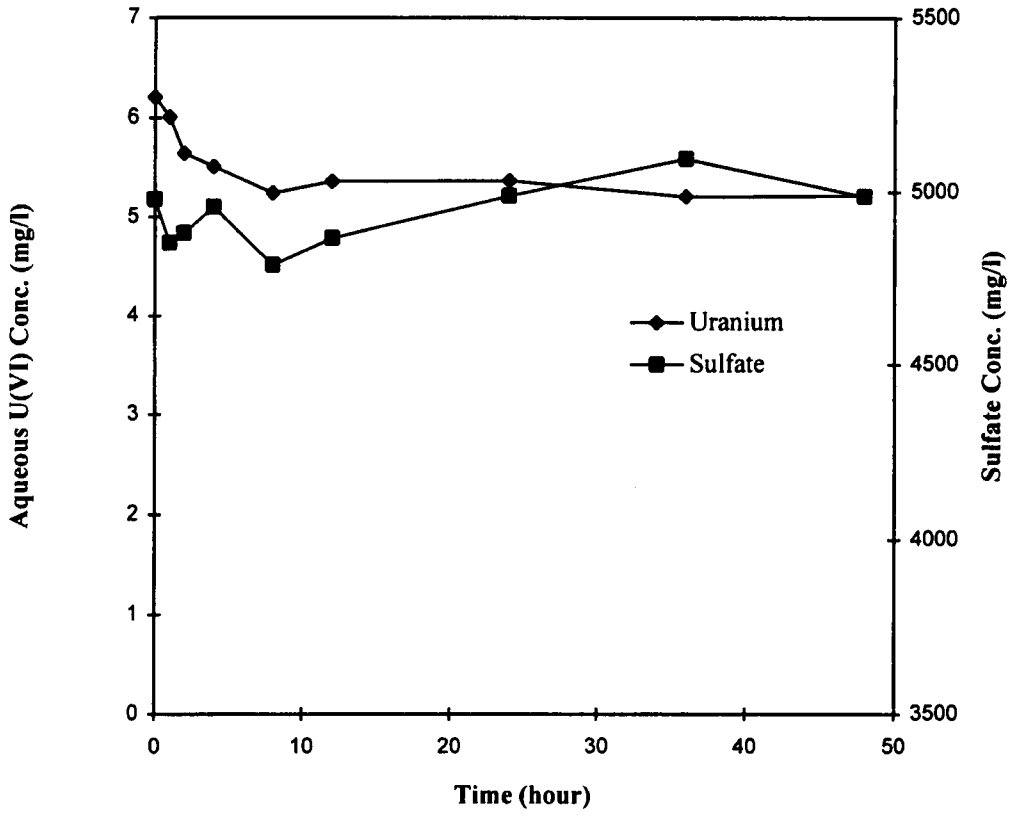

Figure 17. Removal U(VI) and sulfate from the solution containing 10 mg/l copper and 5,000 mg/l sulfate by *D. desulfuricans,* as a function of time. The electron donor was lactate.

amounts of uranium removal in these heavy metal containing matrixes were similar to that of uranium\sulfate (5,000 mg/l) matrix in experiment A. So the concentration of zinc and nickel did not appear toxic in experiment E. Also the results indicate that zinc and nickel were rapidly removed during uranium reduction. The zinc was removed to below the detection limit (0.012 mg/l) in the first hour, and the nickel was removed to below the detection limit (0.12 mg/l) in four hours. However, Figure 17 indicates that the copper concentration in the test solution (8 to 10 mg/l) prevented uranium removal by *D. desulfuricans*. When the uranium solution contains 10 mg/l of copper, neither uranium nor sulfate could be removed by *D. desulfuricans*.

A visual observation in experiment E supported the analytical results by showing that after one day a dark sediment accumulated on the bottom of the reactor tubes for both the zinc and nickel matrixes. At the same time, the solutions became very clear. This result indicated that zinc sulfide and nickel sulfide were formed, and precipitated with U(IV) together. In contrast, the copper matrix did not form the precipitant and the solution remained turbid (suspended organism cells) until the end of the experiment. Also no strong odor of hydrogen sulfide was observed when samples were taken from the copper containing reactor, which indicated that the copper concentration inhibited organism enzymatic activity. Comparing the results for the viable cell reactors with that of the dead cell reactors plotted in Figure 15 and Figure 16, the viable cell reactors could efficiently remove uranium, zinc, and nickel, but the metal concentrations in the dead cell reactors remained almost unchanged during the experiment period. This demonstrated that microbial enzymatic activity was the main reason for uranium, zinc and nickel

removal rather than the biosorption. However, whether or not the indirect enzymatic metal removal mechanism was the main cause of zinc and nickel removal from the solution required further investigation.

Experiment F. This experiment was conducted to investigate the possible mechanism for zinc and nickel removal. There are four possible mechanisms that may cause zinc and nickel removal from the solution. They are direct biological enzymatic removal, metal removal by adsorption to the living cell walls, metal removal by co-precipitate with insoluble U(IV), and indirect biological precipitation by enzymatic sulfate reduction (that is, the biological enzymatic sulfate reduction serves as an indirect reaction to provide sulfide ion for metal-sulfide precipitation).

In order to examine which mechanism is the dominant cause for zinc and nickel removal, sulfate was deleted from the zinc-uranium and nickel-uranium bioreactors in the above experiment E. The results are shown in Table 8, Figures 18 and 19. Table 8 shows that when sulfate was deleted from the solution, uranium removal was unaffected, but zinc and nickel removal were insignificant. This indicated that co-precipitation was not a main mechanism for zinc and nickel removal. Furthermore, Figures 18 and 19 indicate that regardless of whether or not uranium was present in the solution, if sulfate was deficient, zinc and nickel removal were insignificant. These data also indicate that neither direct enzymatic removal nor bio-adsorption are the main removal mechanisms. Therefore, the most probable mechanism for zinc and nickel removal is biological indirect metal-sulfide precipitation.

Table 8. Uranium, zinc, and nickel removal in sulfate-free solutions.

Time	Zinc Test		Nickel Test	
(hour)	U(VI) (mg/l)	Zn (mg/l)	U(VI) (mg/l)	Ni (mg/l)
0	5.482	11.20	5.834	13.12
24	0.242	10.64	0.259	12.05
48	0.197	10.06	0.121	11.52

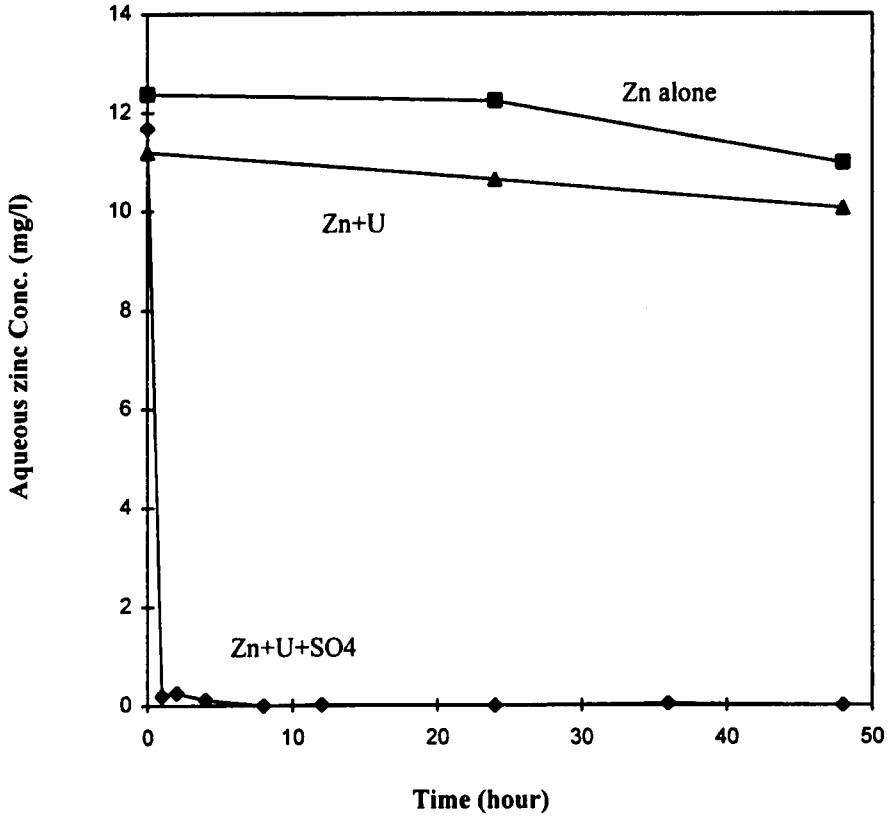

Figure 18. Removal of zinc from the solutions containing 4 mg/l U(VI) or 5,000 mg/l sulfate after inoculation with *D. desulfuricans,* as a function of time.

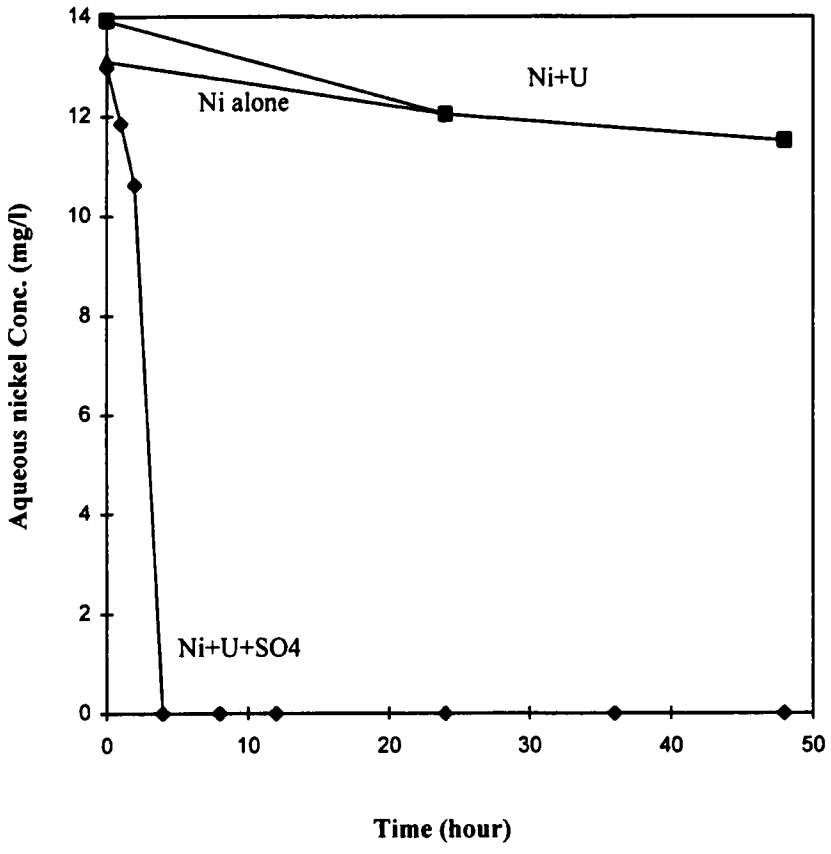

Figure 19. Removal of nickel from the solutions containing 4 mg/l U(VI) or 5,000 mg/l sulfate after inoculation with *D. desulfuricans*,as a function of time.

Experiment G. Since the above experiment E showed that an 8 to 10 mg/l of initial copper concentration could prevent uranium and sulfate reduction by *D. desulfuricans*, this experiment was conducted to further investigate the inhibition of copper for uranium reduction. Four bioreactors were used for this purpose. The initial copper concentration in the four reactors were 1 mg/l, 3 mg/l, 5 mg/l, and 10 mg/l. To simulate Y-12 Plant wastewater, the synthetic wastewater also contained 2,500 mg/l of bicarbonate, 10 mM of lactate, and 5,000 mg/l of sulfate. The results are shown in Figure 20 and Table 9. Figure 20 indicates that copper toxicity for the organism decreased along with the decrease of initial copper concentration. Below initial copper concentrations of 1 mg/l, the inhibition of uranium reduction was insignificant. The rate constants of U(VI) removal (Appendix II) proved also that the rate of U(VI) removal decreased along with the increase of the copper initial concentration. In addition, as the initial copper concentration decreased, sulfate reduction by *D. desulfuricans* increased (Table 9). Subsequently, as the sulfide concentration increased, copper was precipitated from the solution by forming copper-sulfide (CuS) (Table 9). Therefore, sulfide served as a metal detoxificant in the solution (Mizrahi, 1989; Lawrence and McCarty, 1964). This implies that, with an initial copper concentration below 10 mg/l and a longer retention time, the uranium removal could be recovered to a normal level when copper toxicity was controlled by sulfide.

Experiment H. The results of experiment F indicated that the main cause of the selected heavy metals removal from the synthetic wastewater was biological indirect metal-sulfide precipitation. To further prove this mechanism, experiment H was

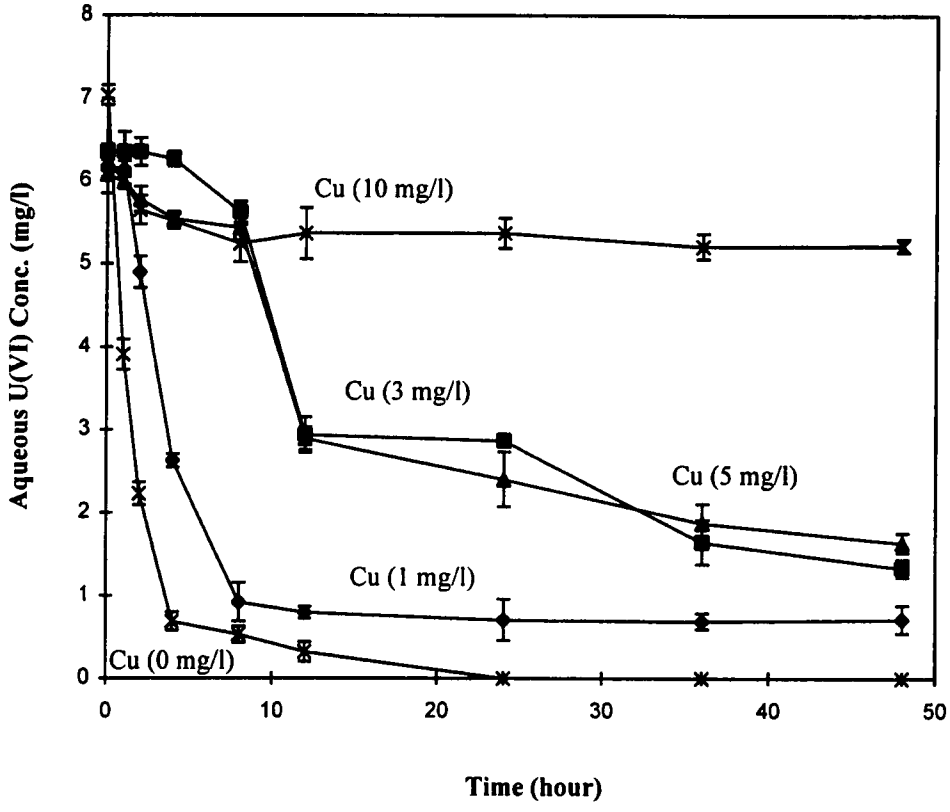

Figure 20. Removal of copper from the solutions after inoculation with *D. desulfuricans,* as a function of time. The electron donor was lactate.

Table 9. Data for different initial copper concentration matrixes

Time	Initial Cu (10 mg/l)		Initial Cu (5 mg/l)		Initial Cu (3 mg/l)		Initial Cu (1 mg/l)	
(hour)	Cu (mg/l)	SO_4 (mg/l)	Cu (mg/l)	SO_4 (mg/l)	Cu (mg/l)	SO_4 (mg/l)	Cu (mg/l)	SO_4 (mg/l)
0	11.70	4979.74	6.18	4885.82	3.86	4531.96	1.24	5522.51
1	8.23	4853.98	3.48	4856.71	1.61	4517.49	0.45	5546.66
2	6.13	4882.55	0.77	4769.78	0.10	4499.44	0.18	5383.01
4	5.51	4657.13	0.13	4797.66	0.08	4297.60	0.13	5479.90
8	5.04	4789.4	0.14	4659.36	0.14	4088.89	0.14	5370.80
12	4.96	4867.22	0.12	4602.63	0.11	4092.37	0.12	5405.11
24	4.24	4989.4	0.08	4617.48	0.09	3845.38	0.12	5235.27
36	4.67	5097.02	0.07	4411.84	0.06	3973.16	0.11	4811.82
48	4.33	4787.8	0.09	4418.29	0.04	3880.83	0.09	4858.28

conducted to compare an indirect enzymatic metal-sulfide precipitation system with an abiotic metal-sulfide precipitation system. Four reactors were used in this experiment. The conditions for this experiment were the same as those for the previous bioreactors: 2,500 mg/l of bicarbonate, 10 mM of lactate, and 12 to 14 mg/l of zinc or nickel. The differences which created an abiotic condition were that sodium sulfide was used to replace sodium sulfate and that there were no organisms in the solutions. Since *D. desulfuricans* can reduce about 250 mg/l of sulfate to sulfide in four hours (experiment A), 83 mg/l of sulfide would be theoretically produced in the solution (Appendix II). Therefore, the amount of sulfide addition in the abiotic experiment was at two levels, 80 mg/l and 30 mg/l. However, these two sulfide levels had already exceeded the amount of sulfide required for zinc (12-14 mg/l) and nickel (12-14 mg/l) precipitation.

A comparison of the abiotic metal-sulfide precipitation reactors (Figure 21 and Figure 22) and the biotreatment reactors (Figure 18 and Figure 19) indicates a difference in metal removal efficiency. The biotreatment reactors achieved almost 100 % metal removal efficiency in a short time (1 hour for zinc and 4 hour for nickel). However, the abiotic reactors could only achieve 33% to 95% removal efficiency for zinc and 37% to 91% removal efficiency for nickel in the test period. An important observation during the abiotic experiment was that the metal sulfide particles could not efficiently be precipitated or filtered. The explanation for this problem could be evaluated by the equilibrium chemistry of the system (Barnett and Robinson, 1991). There are two distinct stages in the process of a new phase formation during precipitation: nucleation and crystal growth (Shaw, 1991). A high degree of dispersion is a result of high

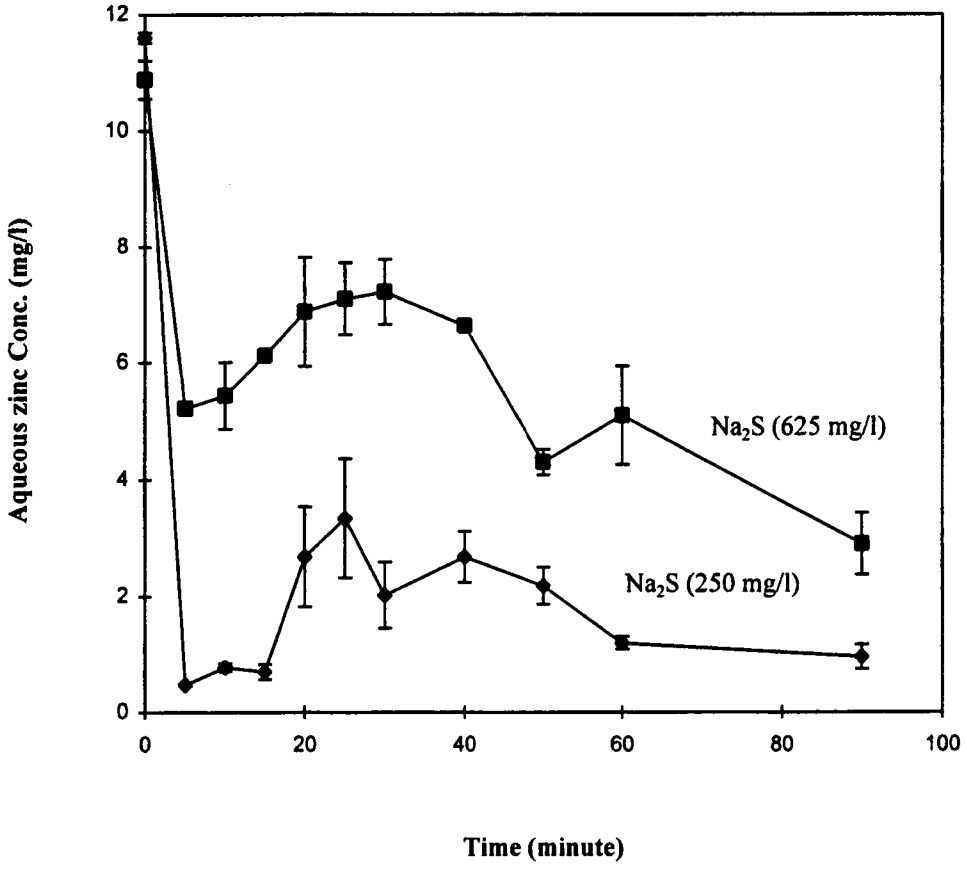

Figure 21. Removal of zinc from abiotic reactors as a function of time. The solutions contained 6 mg/l uranium, 10 mM lactate, and 2,500 mg/l bicarbonate. pH was 7.5.

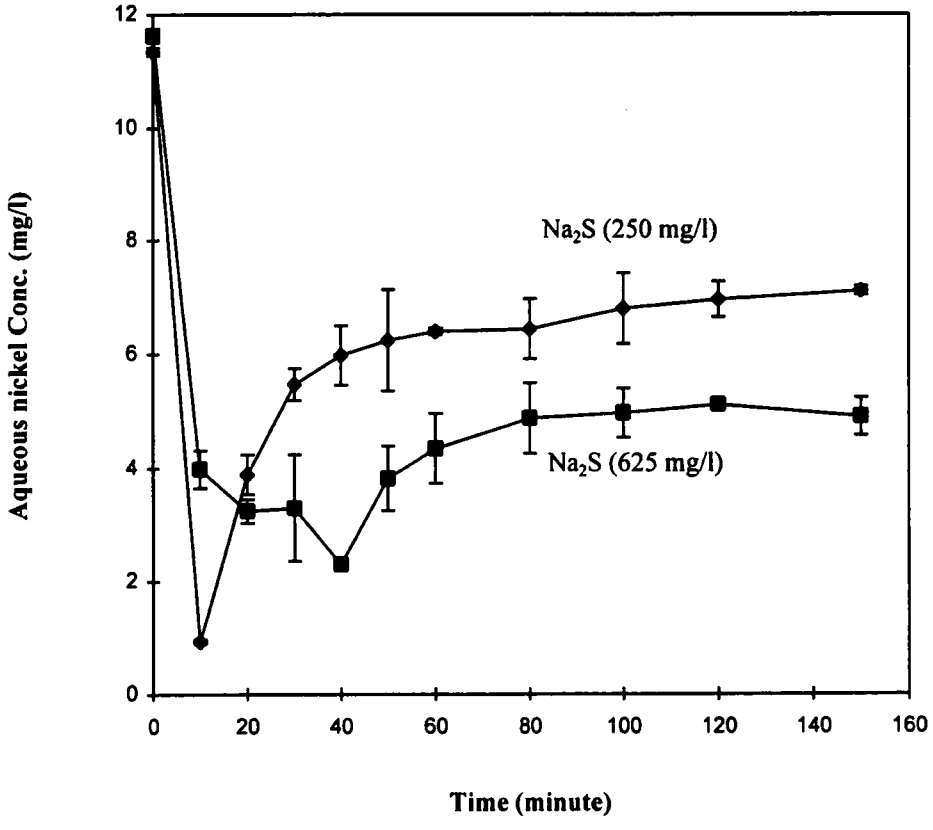

Figure 22. Removal of nickel from abiotic reactors as a function of time. The solutions contained 6 mg/l uranium, 10 mM lactate, and 2,500 mg/l bicarbonate. pH was 7.5.

nucleation rate and low crystal growth rate. The initial rate of nucleation depends on the degree of supersaturation which can be reached before phase separation occurs. The saturation level can be evaluated by saturation index.

$$\text{Saturation Index} = \text{LOG}\frac{[\text{Metal}] \times [\text{Sulfide}]}{\text{Ksp}}$$

Where

[Metal]: Metal ion concentration (M)

[Sulfide]: Sulfide ion concentration (M)

Ksp: Metal sulfide solubility product constant

If the saturation index is greater than 0, the solution is oversaturated with the solid. If the saturation index is less than 0, the solution is undersaturated with solid (Barnett and Robinson, 1991). Most saturation indexes for metal sulfide in the abiotic reactors were higher than 3.0 (from Minteq computer model calculation). This higher saturation index indicated an oversaturation condition in the test solution. The oversaturation could slow down the rate of crystal growth, hence, make the nucleation relatively fast. As a result of this, many small particles were formed in the solution, which made the solid-liquid separation difficult. This has been found to be a problem in the treatment of metal finishing wastes by sulfide (EPA, 1980). To solve this problem, one common suggestion is to use an insoluble iron-sulfide slurry to replace soluble sodium-sulfide, since an iron-sulfide slurry can control sulfide ion concentration at

certain low levels (EPA, 1980); The second common suggestion is to add a coagulant aid to form and maintain a concentrated sludge blanket, since this sludge blanket can catch dispersed small metal-sulfide particles (Shlauch, 1977).

The Minteq geochemical equilibrium model was run to determine the theoretical complexation of the metal species involved in experiment H. At the abiotic experimental condition, the heavy metals could not be efficiently precipitated from the solution, so the computer model utilized "not allowing precipitation" as the running condition. According to theoretical calculation, if metal species can not be separated from the solution promptly, they must be complexed in species, such as $Zn(HS)_3^-$, $NiCO_3(AQ)$, and $Cu(HS)_3^-$, in the abiotic reactors. The formation of these metal complexes could increase the concentration of soluble metal.

Either of these two reasons, oversaturation or metal-complex species, could cause lower metal removal efficiency in the abiotic reactors.

According to the discussion above, therefore, a biotreatment system may have advantages over an abiotic system based on the followings:

1. In biotreatment systems the organisms act as the insoluble iron-sulfide slurry to gradually release sulfide ion into the solution. This controls the sulfide concentration at a level to balance the crystal growth and nucleation process. As a result, metal sulfide oversaturation may be decreased, and solid-liquid separation may be more efficient.

2. Biomass may intercept small metal-sulfide particles and make them easy to separate from the solution.

These advantages may make coagulants, flocculants, and filtration, which are normally required by the abiotic metal-sulfide precipitation system, unnecessary in the biotreatment system.

However, concurrent removal of uranium and other heavy metals by *D. desulfuricans* also has potential disadvantages. One of the advantages for uranium removal in a *D. desulfuricans* system is its low sludge production, since uranium as the electron acceptor does not sufficiently support organism growth. If the uranium contaminated wastewater also contains high sulfate concentration, the sulfate reduction would greatly support organism growth (Lovley and Phillips, 1992; Kucsmas, 1994), which may cause a high sludge production. In addition, the metal-sulfide products would cause an extra amount of sludge. This also may cause a higher sludge production than that of a single enzymatic uranium reduction system. Therefore, the storage of this uranium contaminated sludge may be a concern. Furthermore, sulfide, which is the product of enzymatic sulfate reduction, is also a corrosive and toxic compound. It is toxic to fish, exerts an oxygen demand, and has the potential for serious odor problem. So high level of sulfide production may cause corrosion in the treatment system and discharge problems (Singh, 1992).

4.4. Bioprecipitation of uranium and other heavy metals in solutions containing organic chelating agents

Unknown organic chelating agents in the Y-12 Plant wastewater was another important factor that may affect uranium enzymatic reduction and the selected heavy metals precipitation in the *D. desulfuricans* system. These organic chelating agents would

complex with uranium and selected metals, which may make them unavailable for direct enzymatic reduction and indirect metal-sulfide precipitation.

To evaluate this effect, experiments were conducted with representative organic chelating agents. However, bicarbonate, an inorganic chelating agent, was in the Y-12 Plant wastewater and the synthetic wastewater. The previous experimental results proved that the bicarbonate did not inhibit uranium reduction or any selected heavy metals precipitation in the synthetic wastewater. Citrate and EDTA were selected as representative organic chelating agents. The stability constant of metal-citrate complex is lower than that of metal-bicarbonate complex and the stability constant of metal-EDTA complex is higher than that of metal-bicarbonate complex. Hence, citrate was used to represent the organic ligands weaker than bicarbonate, while EDTA was used to represent the organic ligands stronger than bicarbonate. According to a Minteq program calculation, in the experimental conditions, the stability constants (log K) of zinc-citrate and nickel-citrate were 8.98 and 6.76, respectively. Log K of zinc-EDTA and nickel-EDTA were 16.95 and 20.84, respectively. Finally, Log K of zinc-bicarbonate and nickel-bicarbonate were 12.5 and 12.6. respectively.

The synthetic wastewater for this experiment was prepared in the same way as in the previous metal removal experiments. Six bioreactors were used in the experiment: a uranium-citrate reactor, a uranium-EDTA reactor, a uranium-zinc-citrate reactor, a uranium-zinc-EDTA reactor, a uranium-nickel-citrate reactor, and a uranium-nickel-EDTA reactor. The amount of chelating agent added was computed by the Minteq geochemical equilibrium model to ensure 100% chelation of zinc and nickel. Since there were no

stability constants for the uranium-citrate and uranium-EDTA complexes in this model and since this research concentrated on the precipitation of zinc and nickel sulfides from organic ligand solutions, uranium complexation was not evaluated.

An important observation noted that at pH 7.0 to get 100% metal-citrate complex in the presence of bicarbonate, more than 0.02 M citrate was needed. Since bicarbonate was a stronger ligand than citrate, metal-citrate complexes were not easily formed. In contrast, to get 100% metal-EDTA complex in the presence of bicarbonate, only 0.0005 M EDTA was needed. Since bicarbonate was a weaker ligand than EDTA, metal-EDTA complexes were more easily formed than metal-citrate complexes. This observation demonstrated that bicarbonate had an important influence on metal-ligand complexes formation in the test conditions. Therefore, using the stability constant of bicarbonate as a reference to choose chelating agents for this experiment was reasonable.

Figures 23, 24, and 25 indicate that citrate caused a decrease of uranium, zinc and nickel removal, when compared with the non-citrate condition (experiment E). For the zinc-citrate reactor, uranium removal efficiency decreased from the non-citrate removal efficiency of 79.1 % to 30.9 % over 48 hours. Zinc removal efficiency did not appear to change significantly, but there was a certain low level residual that could not be removed at a short time. For the nickel-citrate reactor, uranium removal efficiency decreased from the non-citrate removal efficiency of 84.4 % to 38.4 % over 48 hours. Likewise, the nickel removal efficiency decreased from 100 % to 14.1 % over 4 hours. The calculations for the metal removal rate constants (Appendix II) also indicated that metal removal process was slower in a citrate solution than in a non-citrate solution. The uranium removal rate

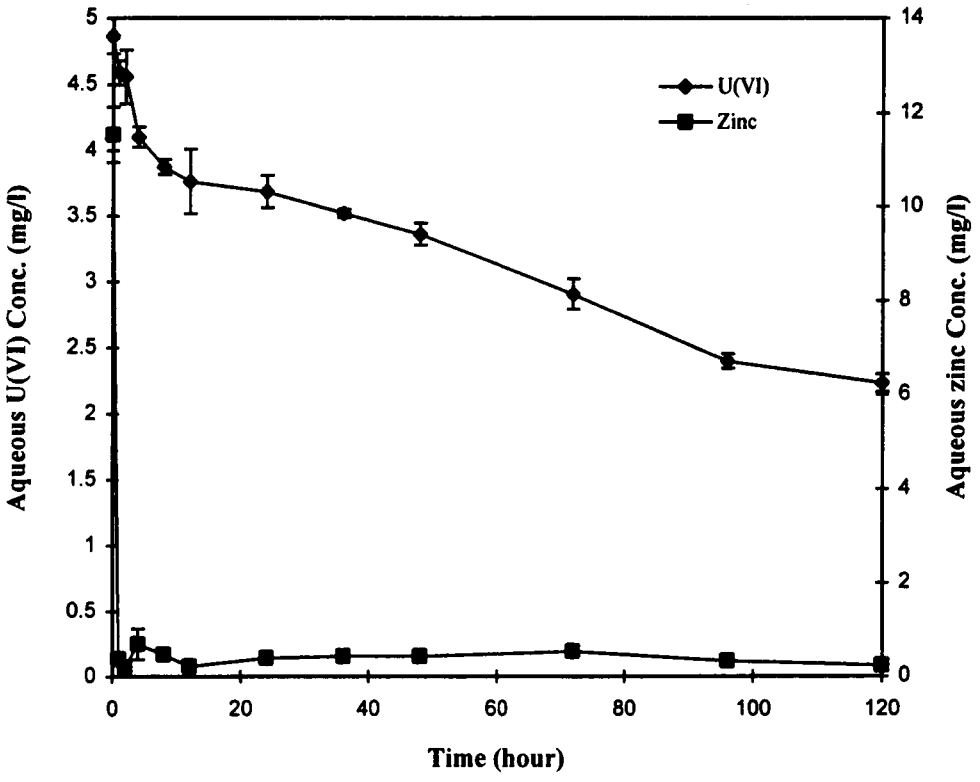

Figure 23. Removal of U(VI) and zinc from citrate solution after inoculation with *D. desulfuricans,* as a function of time. The solution contained 0.02 M citrate and 5,000 mg/l sulfate. The electron donor was lactate.

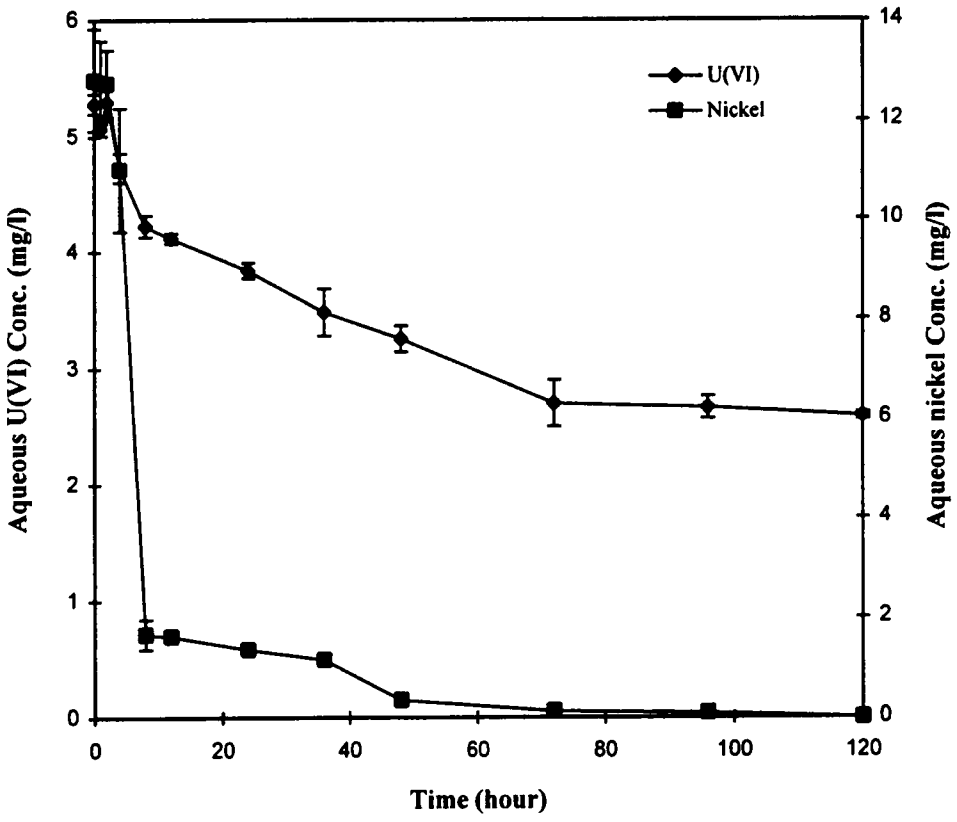

Figure 24. Removal of U(VI) and nickel from citrate solution after inoculation with *D. desulfuricans,* as a function of time. The solution contained 0.02 M citrate and 5,000 mg/l sulfate. The electron donor was lactate.

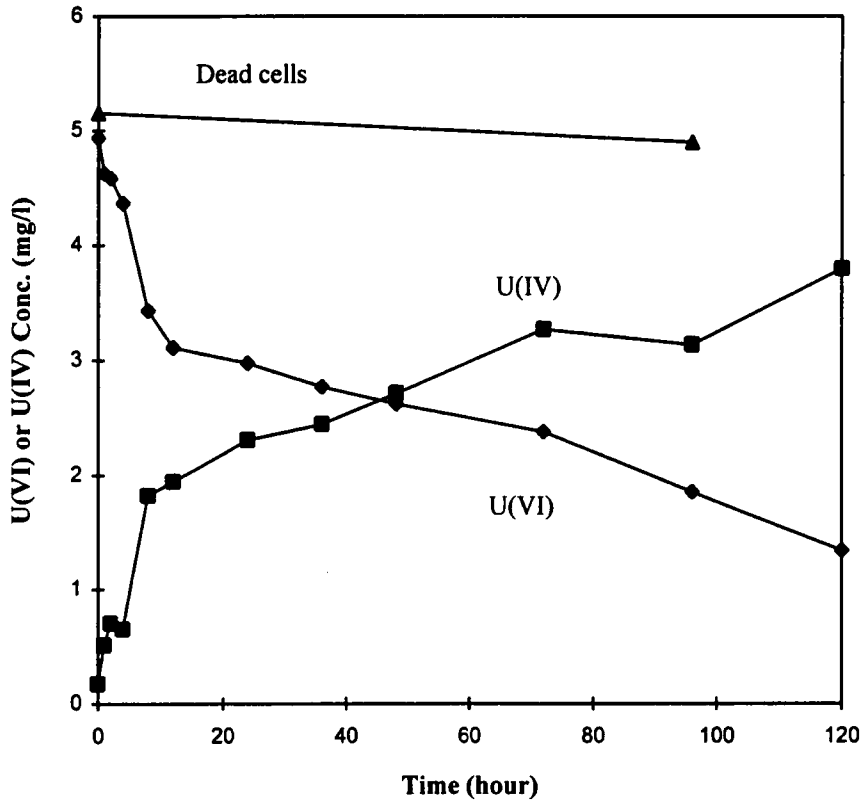

Figure 25. Reduction of U(VI) to U(IV) in citrate solution by *D. desufuricans*, as a function of time. The solution contained 0.02 M citrate and 5,000 mg/l sulfate. The electron donor was lactate.

constant decreased from 0.3113 hr^{-1} in a non-citrate solution to 0.0091 hr^{-1} in a citrate solution. The zinc removal rate constant decreased from 1.0617 hr^{-1} in a non-citrate solution to 0.8045 hr^{-1} in a citrate solution. The nickel removal rate constant decreased from 0.1002 hr^{-1} in a non-citrate solution to 0.0568 hr^{-1} in a citrate solution. An experimental observation was that the dark color (a color of zinc sulfide and nickel sulfide) in a citrate solution appeared more slowly than that in a non-citrate solution, which further indicated that metal sulfide precipitation process in a citrate solution was slower than that in a non-citrate solution. Three reasons could cause this apparent process declination:

(1) Free metal cations were slowly released from the metal-citrate complex, which made less free metal ions available in the solution for sulfide precipitation.

(2) Citrate ions were adsorbed onto the precipitate surface which depressed the crystal growth rate (Patterson, 1989).

(3) Metal-citrate complexes were toxic to *D. desulfuricans*.

Figure 25 shows that in the dead cell reactor, almost no uranium removal was observed. It indicated that uranium removal was the result of bioactivity of *D. desulfuricans*. Also the concurrent formation of U(IV) with the U(VI) removal indicated that the main cause of uranium removal from this citrate containing solution was enzymatic uranium reduction. According to the literature (Francis et al., 1992; Francis and Dodge, 1993), metal complexation is a main factor that affects the biodegradation of metal-citrate complexes. Uranium-citrate complexation depends on the pH value and the concentration of uranium and citrate in the solution. However, since this research did not concentrate on

uranium complexation, uranium bioreduction in complexed form was not evaluated in detail. To investigate this objective, a further research is required.

The three possible mechanisms that could lead to uranium reduction and zinc and nickel precipitation from metal-citrate complexes are:

(1) *D. desulfuricans* could directly enzymatically reduce U(VI) from these complexes.

(2) *D. desulfuricans* could use citrate as an energy source. As citrate was biodegraded, uranium and selected metals were released from metal-citrate complexes and became available for the reduction and precipitation reactions.

(3) *D. desulfuricans* could not biodegrade citrate. Reduction and precipitation reactions could only occur due to the chemical equilibrium moving from one compound to another (Shuttleworth and Unz, 1991; Madsen and Alexander, 1985).

Since the literature indicates that *D. desulfuricans* can not use citrate as a energy source, the uranium reduction mechanism in this test solution seems to be *D. desulfuricans* directly reducing U(VI) to U(IV) from uranium-citrate complexes. Also, zinc and nickel removal mechanisms may be the chemical equilibrium moving from metal-citrate complex to metal-sulfide precipitation.

Figures 26 and 27 indicate that EDTA totally inhibited uranium reduction and precipitation of zinc-sulfide and nickel-sulfide. The reasons for this inhibition could be:

(1) EDTA or metal-EDTA complexes were toxic to *D. desulfuricans*.

(2) Metal-EDTA complexes had the higher stability constant, which hindered heavy metals from sulfide precipitation.

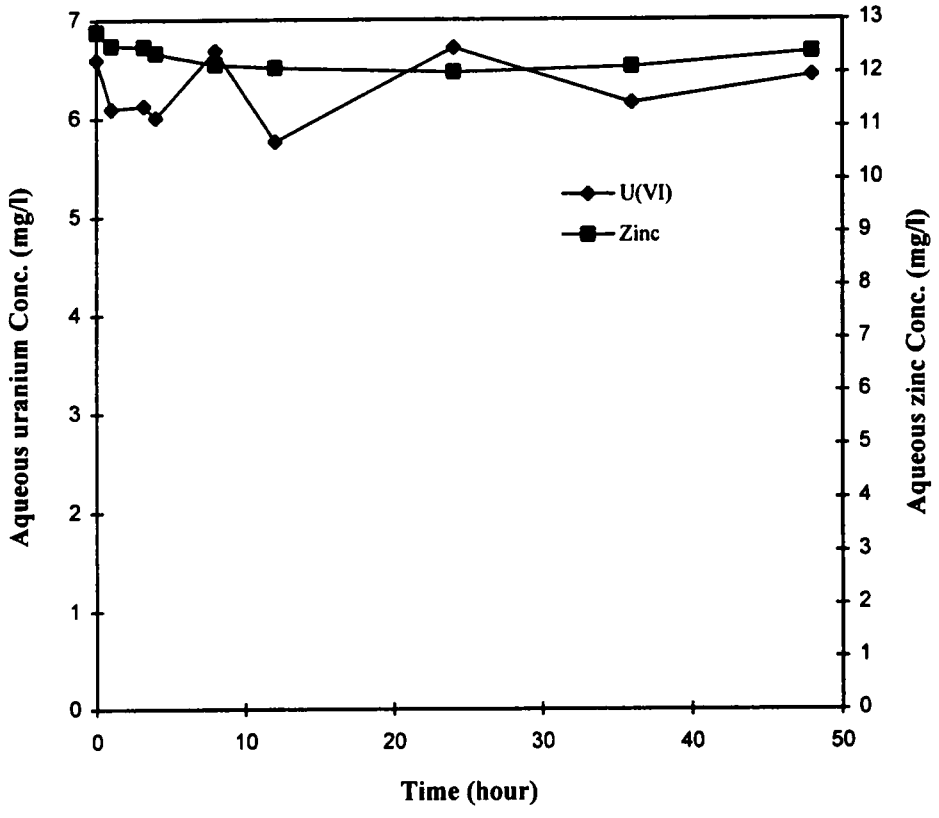

Figure 26. U(VI) and zinc levels in EDTA solution after inoculation with *D. desulfuricans,* as a function of time. The solution contained 0.0005 M EDTA and 5,000 mg/l sulfate. The electron donor was lactate.

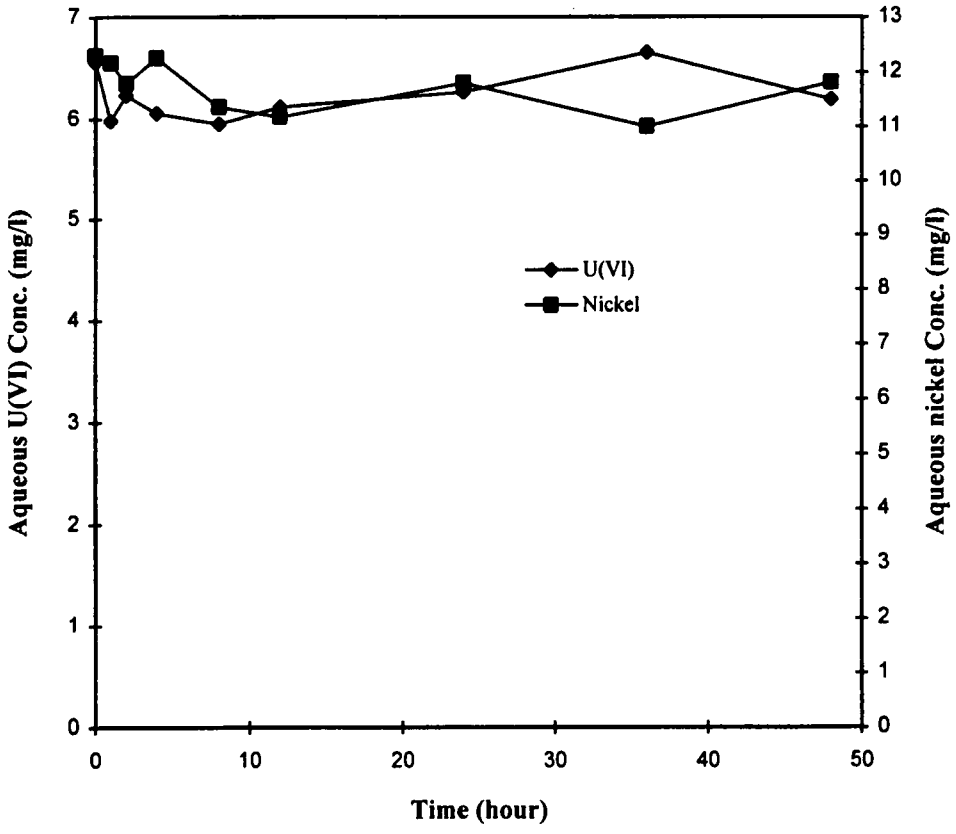

Figure 27. U(VI) and nickel levels in EDTA solution after inoculation with *D. desulfuricans,* as a function of time. The solution contained 0.0005 M EDTA and 5,000 mg/l sulfate. The electron donor was lactate.

(3) Uranium was not biologically available in uranium-EDTA complexes.

Figure 28 indicates that EDTA, as well as its metal complexes did not stop *D. desulfuricans* activity in these experimental conditions because sulfate reduction was not inhibited. Therefore, the reasons for EDTA inhibition were the higher stability constant of metal-EDTA complexes and the bioavailability of the uranium-EDTA complexes.

The above results suggest a rough way to evaluate the effect of organic chelating agents on the *D. desulfuricans* system: if the stability constant of a metal-ligand complex is smaller than that of the metal-bicarbonate complex, uranium reduction and metal precipitation by sulfide may occur; if the stability constant of a metal-ligand complex is larger than that of the metal-bicarbonate complex, uranium reduction and metal precipitation may fail.

4.5. Y-12 Plant wastewater experiment

To investigate whether *D. desulfuricans* could work in a more complicated wastewater matrix, a Y-12 Plant wastewater bench experiment was conducted as a final step of this research. Since it was unknown whether or not the Y-12 Plant wastewater contained any potential interference on the uranium analysis, a standard addition method was used to ensure accuracy of uranium analysis. The standard addition curve is plotted in Figure 29. The results of the standard addition indicate that the Y-12 Plant wastewater did not cause interference on the uranium analysis. Table 5 indicates that tank 3 contained higher pollutant levels than the others, so it was chosen as the test solution in the Y-12 plant wastewater experiment. The only chemical addition was lactate (10 mM) as an electron donor. After *D. desulfuricans* was injected into the Y-12 plant wastewater reactors, the

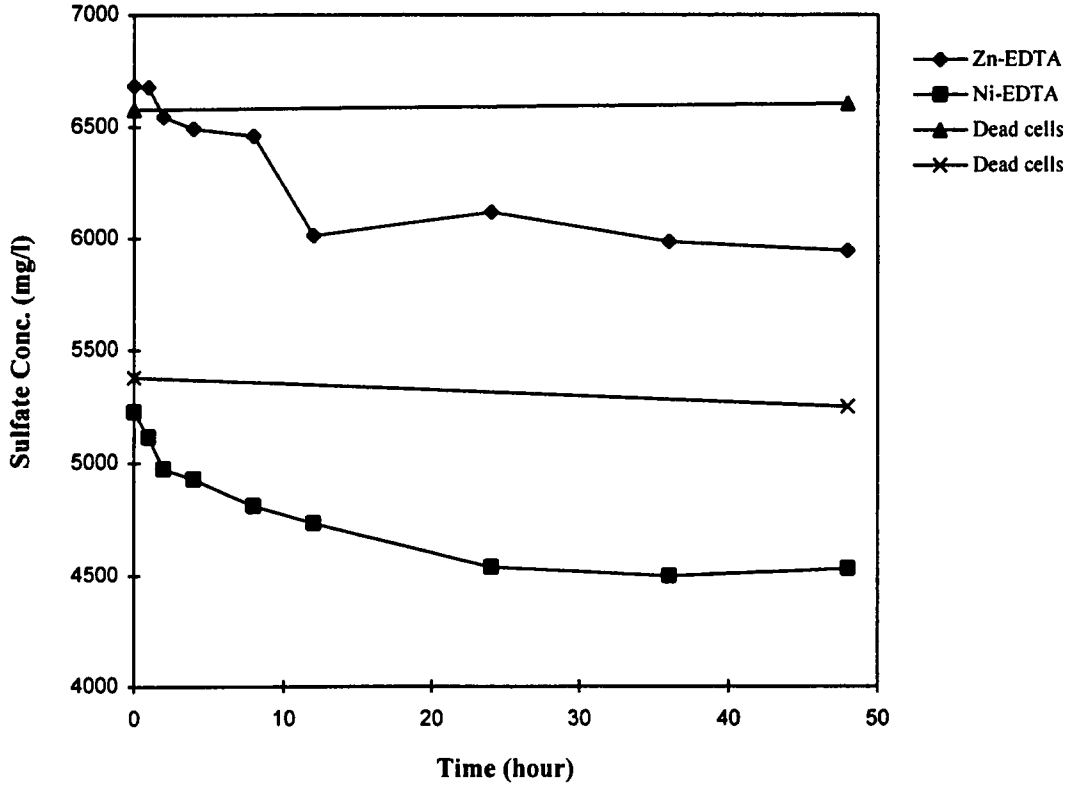

Figure 28. Removal of sulfate from Zn-EDTA or Ni-EDTA complex solutions as a function of time. The solutions contained 0.0005 M EDTA, 12 mg/l zinc (or nickel), and 5,000 mg/l sulfate. The electron donor was lactate.

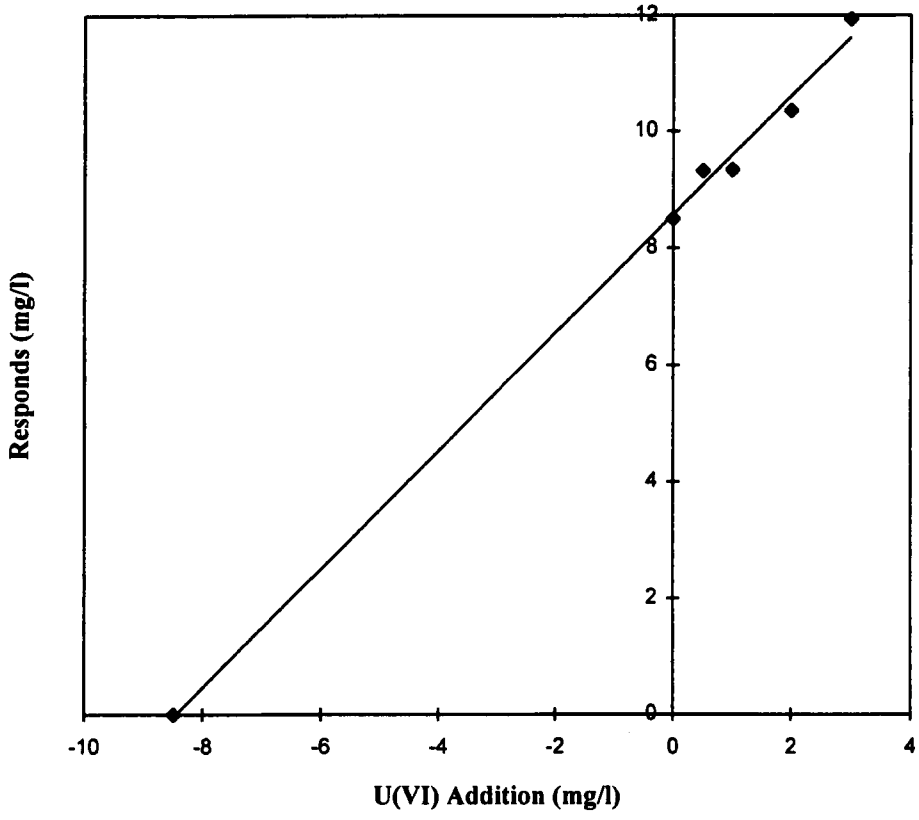

Figure 29. Standard addition curve for tank #3 wastewater from Y-12 Plant.

samples from the viable cell reactors were taken at 0, 1, 2, 4, 8, 12, 24, 36, and 48 hours, and the samples from the dead cell reactors were taken at 0, 12, 30, and 48 hours.

The results plotted in Figures 30 and 31 indicate that uranium reduction efficiency was around 50%. For the selected heavy metals, removal efficiencies were approximately 100% for zinc, 40% for nickel, and 64% for copper during the test period (Figure 31). The dead cell data indicated that uranium and selected heavy metals removal were caused by organism activity. When comparing the results of the Y-12 Plant wastewater experiment to that of synthetic wastewater experiments, three inhibition factors could be neglected:

(1) The Y-12 Plant wastewater was not toxic to *D. desulfuricans*, since sulfate reduction was going on during the experiment period (Figure 30).

(2) Copper concentration in Y-12 Plant wastewater was lower than 1 mg/L, so it should not have significant impact on *D. desulfuricans* enzymatic activity.

(3) Ionic strength inhibition due to sulfate ion could not exist because sulfate concentration in the Y-12 Plant wastewater was below 5000 mg/L.

Comparing the rate constants of the heavy metal removal in the Y-12 Plant wastewater with that in the synthetic wastewater (Appendix II), the rates of the heavy metal removal were slower in the Y-12 Plant wastewater than in the synthetic wastewater. The uranium removal rate constant decreased from 0.0595 hr^{-1} in the synthetic wastewater to 0.0149 hr^{-1} in the Y-12 Plant wastewater. The zinc removal rate constant decreased from 1.0617 hr^{-1} in the synthetic wastewater to 0.9391 hr^{-1} in the Y-12 Plant wastewater. The nickel removal rate constant decreased from 0.1002 hr^{-1} in the synthetic wastewater to

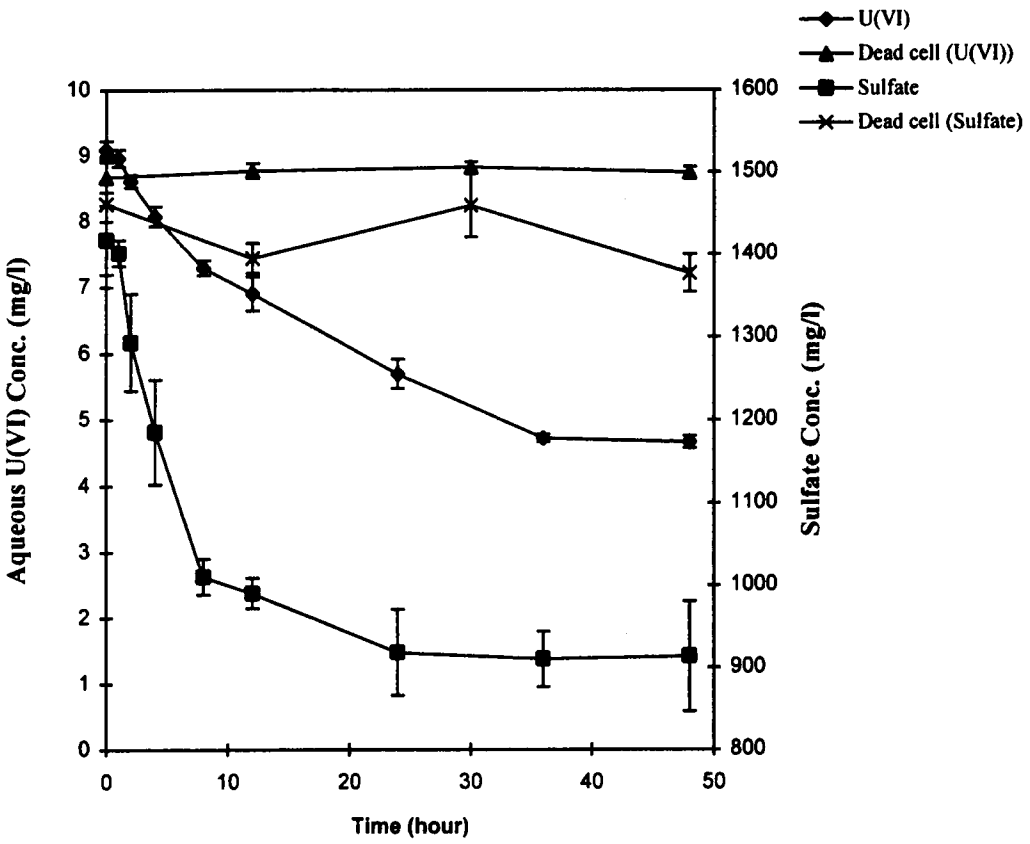

Figure 30. Removal of U(VI) and sulfate from Y-12 Plant wastewater after inoculation with *D. desulfuricans,* as a function of time. The electron donor was lactate (10 mM).

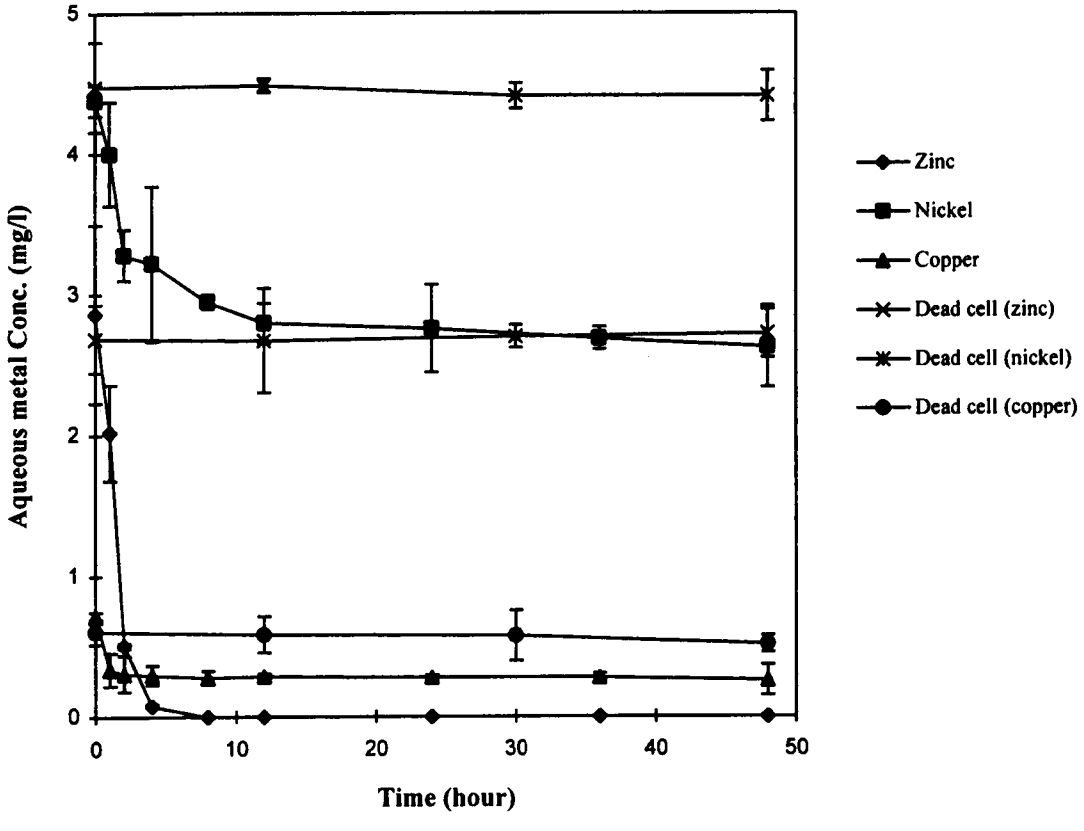

Figure 31. Removal of heavy metals from Y-12 Plant wastewater after inoculation with *D. desulfuricans,* as a function of time. The electron donor was lactate (10 mM).

0.0848 hr^{-1} in the Y-12 Plant wastewater. The possible explanations for this declination were:

(1) The ionic strength of the Y-12 Plant wastewater was unknown. Therefore, a possible reason that caused the metal removal declination from the Y-12 Plant wastewater could be some unknown ions which made ionic strength in the solution higher than in the synthetic wastewater, so uranium reduction was delayed.

(2) The Y-12 Plant wastewater could have contained certain organic or inorganic ligands that were stronger than citrate but weaker than EDTA, especially for the nickel-ligand complex. This reason is based on the chemical equilibrium mechanism for uranium reduction and metal precipitation in the presence of citrate. The stability constant of the metal-ligand complex could have been too strong for the metal to be precipitated easily by sulfide.

(3) Also there could be some strong organic or inorganic ligands similar to EDTA in the wastewater, which complexed so tightly with uranium and the selected metals that uranium and the selected metals were not available for enzymatic reduction and metal-sulfide precipitation by *D. desulfuricans* bioactivity.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Concurrent removal of uranium and selected heavy metals by *D. desulfuricans* was evaluated at the bench scale. Four factors that possibly affect the system efficiency were investigated: uranium reduction at low initial concentration, uranium reduction in a sulfate solution, concurrent removal of uranium and selected heavy metals, and the effect of chelating agents.

(1) Enzymatic uranium reduction at low initial concentration is possible, but U(VI) removal will be slower than at high initial concentration. According to the results in this study, the retention time for a biotreatment facility should be longer than one day to achieve the greatest uranium removal efficiency at low initial concentration of uranium (4-6 mg/l). The uranium reduction rate was approximated by first order kinetics at this low initial concentration.

(2) Under the experimental conditions, when sulfate concentration was increased from 0 to 10,000 mg/l, the effectiveness of *D. desulfuricans* for uranium removal may decrease. This decrease might be caused by a combined effect of high ionic strength, electron acceptor competition, and electron donor limitation. In a low ionic strength condition, the effect of sulfate concentration appears to be more important than that of ionic strength. In a high ionic strength condition, the effect of ionic strength appears to be more important than that of sulfate concentration.

(3) Selected heavy metal precipitation by sulfide are also possible in the *D. desulfuricans* system. Under the experimental conditions, the removal efficiencies of selected heavy metals in the biotreatment experiment were higher than in the abiotic experiment, because the biotreatment system could lower the oversaturation level of metal sulfide in the solution and achieve more efficient metal-sulfide settling. In the synthetic wastewater, zinc and nickel could be removed by indirect enzymatic sulfide precipitation at approximately 100% efficiency within one hour and four hours, respectively. Copper is toxic to *D. desulfuricans*. The influent concentration of copper should be controlled at a concentration lower than 1 mg/l for the greatest uranium removal efficiency. Either a pretreatment facility may be needed to lower the influent concentration of copper, or the system retention time should be extended, since sulfide in the solution could serve as a detoxificant to lower the copper concentration with time.

(4) Organic and inorganic ligands in the wastewater should be clearly understood. Factors involved in this understanding include their toxicity, metal-ligand complex structure, biodegradability, and stability constant for the complex. According to the experimental results, in a bicarbonate solution, if the stability constant of a metal-ligand complex is smaller than that of the metal-bicarbonate complex, metal-sulfide precipitation may occur; if the stability constant of a metal-ligand complex is larger than that of the metal-bicarbonate complex, metal-sulfide precipitation may fail. Further research is needed to investigate the behavior of a variety of organic and inorganic chelating agents in wastewater, especially for those ligands in the Y-12 Plant wastewater. This further research

could provide the information needed to determine which chelating agent should be limited to maintain *D. desulfuricans* system operation.

(5) Experimental analysis and observation indicated that the product of the enzymatic reduction, U(IV), can be oxidized back to U(VI) when exposed to oxygen before sufficiently settled in anaerobic conditions. So an anaerobic secondary settling facility may be needed to ensure that most of the insoluble U(IV) settles out and does not reenter the effluent solution as soluble U(VI).

(6) For the given Y-12 Plant wastewater, the experimental results demonstrate that the uranium reduction efficiency, the zinc, nickel, and copper removal efficiency were approximately 50%, 100%, 40%, and 64%, respectively. With appropriate control of the investigated affecting factors, the *D. desulfuricans* biotreatment method is a potentially effective post-treatment technology for the Y-12 Plant and similar industrial wastewaters.

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APPENDIXES

APPENDIX I

Analytical Results

Uranium Mass Balance Results

Time (hr)	U(VI)-unfiltered (mg/l)	U(VI)-filtered (mg/l)	Total uranium-unfiltered (mg/l)	Total uranium-filtrate (mg/l)
0	4.348	4.827	4.657	4.824
1	2.92	2.771	4.573	3.619
2	1.521	1.365	4.337	3.258
4	0.582	0.433	4.65	2.131
8	0.398	0.227	4.533	1.321
12	0.325	0.1	4.561	0.923
24	0.198	0	4.659	0.1
36	0.131	0	4.571	0

Lower and High Initial Uranium Concentration Matrix Results

U (4-10 mg/l)		U (200 mg/l) (Lovely)		Kill cell	
time (hr)	U (mg/l)	Time (hr)	U (mg/l)	Time (hr)	U (mg/l)
0	7.04	0	228.48	0	7.4
1	3.91	1	73.78	48	7.52
2	2.23	2.2	40.46		
4	0.69	3.7	2.38		
8	0.53	5.8	0		
12	0.32	12	0		
24	0	24			
36	0	36			
48	0	48			

Sulfate Matrixes Results (containing 10 mM lactate)

U+SO4(0mg/l)		U+SO4(500mg/l)		U+SO4(1000mg/l)	
Time (hr)	U (mg/l)	Time (hr)	U (mg/l)	Time (hr)	U (mg/l)
0	7.04	0	5.875	0	5.76
1	3.91	1	5.643	1	5.589
2	2.23	2	3.607	2	4.351
4	0.69	4	1.843	4	2.891
8	0.53	8	0.82	8	1.21
12	0.32	12	0.535	12	0.975
24	0	24	0	24	0.809
36	0	36	0	36	0.699
48	0	48	0	48	0

U+SO4(5000mg/l)		U+SO4(10000mg/l)	
Time (hr)	U (mg/l)	Time (hr)	U (mg/l)
0	5.576	0	5.968
1	5.525	1	5.894
2	5.325	2	5.54
4	4.9	4	5.03
8	4.13	8	4.149
12	3.11	12	3.03
24	2.469	24	2.313
36	1.985	36	2.296
48	1.798	48	2.237

Kill cell (0 mg/l SO4)		Kill cell (10000 mg/l SO4)	
Time (hr)	U (mg/l)	Time (hr)	U (mg/l)
0	7.4	0	5.82
48	7.52	48	5.79

Same Ionic Strength Matrixes Results

1. U+SO4(10000 mg/l)		2. U+ SO4(500 mg/l)+ NO3	
Time (hr)	Uranium (mg/l)	Time (hr)	Uranium (mg/l)
0	5.968	0	6.594
1	5.894	1	6.391
2	5.54	2	6.177
4	5.03	4	5.178
8	4.149	8	3.752
12	3.03	12	3.272
24	2.313	24	2.21
36	2.296	36	1.78
48	2.237	48	1.2

Sodium Toxicity Matrixes Results

1. U+NaNO3(6448 mg/l NO3)		2. U+Na2SO4 (5000 mg/l SO4)	
Time (hour)	Uranium (mg/l)	Time (hour)	Uranium (mg/l)
0	5.871	0	5.576
1	5.051	1	5.525
2	3.708	2	5.325
4	1.987	4	4.9
8	0.725	8	4.13
12	0.553	12	3.11
24	0.396	24	2.469
36	0.116	36	1.985
48	0.129	48	1.798

Sulfate Matrixes Results (2 mM lactate)

U+SO4 (0 mg/l)				U+SO4 (500 mg/l)	
Time (hr)	U (mg/l)	Kill cell Time (hour)	U (mg/l)	Time (hr)	U (mg/l)
0	6.404	0	6.02	0	6.09
1	3.858	48	6.45	1	4.17
2	2.167			2	3.626
4	1.335			4	2.011
8	0.397			8	1.356
12	0.12			12	1.008
24	0			24	0.956
36	0			36	0.882
48	0			48	0.368

U+ SO4 (1000 mg/l)		U+SO4(5000 g/l)			
				Kill cell	
Time (hr)	U (mg/l)	Time (hr)	U (mg/l)	Time (hr)	U (mg/l)
0	6.053	0	5.944	0	6.26
1	5.358	1	5.820	48	5.92
2	4.087	2	5.487		
4	2.313	4	5.326		
8	0.936	8	4.358		
12	0.652	12	3.846		
24	0.624	24	3.023		
36	0.555	36	2.349		
48	0.000	48	1.528		

Sulfate Matrixes Results (sulfate data)

10mM lactate				2mM Lactate			
Time (hr)	SO4-2 (mg/l) 500(mg/l)	1000(mg/l)	5000(mg/l)	Time (hr)	SO4-2 (mg/l) 500(mg/l)	1000(mg/l)	5000(mg/l)
0	462.62	935.99	5032.21	0	527.02	957.01	5302.46
1	447.85	880.62	4963.65	1	388.21	882.74	5244.48
2	353.7	805.98	4674.78	2	381.37	839.74	5103.1
4	325.7	793.52	4564.13	4	380.38	820.43	4973.12
8	155.34	588.21	4499.72	8	370.12	789.46	4998.51
12	82.98	444.62	4458.49	12	343.58	792.67	4876.94
24	44.11	442.3	4063.39	24	330.42	785.73	4877.11
36	25.59	371.19	4196.89	36	346.25	767.09	4759.17
48	0	385.94	4062.34	48	270.29	764.02	4419.68

Uranium\Sulfate\Zinc or Nickel Matrixes Results

U+Ni+SO4(5000mg/l)			Ni alone		Ni+U		
Time (hr)	U (mg/l)	Ni (mg/l)	Time (hr)	Ni (mg/l)	Time (hr)	U (mg/l)	Ni (mg/l)
0	6.205	12.99	0	13.93	0	5.834	13.115
1	5.558	11.86	24	12.05	24	0.321	12.05
2	5.486	10.63	48	11.515	48	0.259	11.515
4	4.827	0					
8	3.091	0					
12	2.294	0					
24	1.329	0					
36	1.273	0					
48	0.97	0					

U+Zn+SO4(5000mg/l)			Zn alone		Zn+U		
Time (hr)	U (mg/l)	Zn (mg/l)	Time (hr)	Zn (mg/l)	Time (hr)	U (mg/l)	Zn (mg/l)
0	6.16	11.68	0	12.38	0	5.482	11.2
1	5.9	0.2	24	12.24	24	0.242	10.64
2	5.08	0.26	48	10.98	48	0.197	10.06
4	3.88	0.12					
8	3.14	0					
12	2.53	0.02					
24	1.78	0					
36	1.47	0.04					
48	1.29	0					

Kill cell			Kill cell		
Time (hr)	U (mg/l)	Ni (mg/l)	Time (hr)	U (mg/l)	Zn (mg/l)
0	6.16	15.03	0	6.5	12.32
24	6.18	14.97	24	6.36	11.94
48	6.01	15.06	48	6.22	12.08

Uranium\ Sulfate\ Copper Matrixes Results

U+Cu (1mg/l)			U+Cu (3mg/l)		
Time (hr)	U (mg/l)	Cu (mg/l)	Time (hr)	U (mg/l)	Cu (mg/l)
0	6.178	1.235	0	6.349	3.855
1	6.143	0.445	1	6.351	1.61
2	4.897	0.175	2	6.35	0.095
4	2.629	0.13	4	6.264	0.08
8	0.922	0.135	8	5.619	0.14
12	0.794	0.115	12	2.937	0.11
24	0.705	0.12	24	2.866	0.085
36	0.69	0.11	36	1.646	0.055
48	0.713	0.085	48	1.332	0.04

U+Cu (5mg/l)			U+Cu (10mg/l)			
Time (hr)	U (mg/l)	Cu (mg/l)	Time (hr)	U (mg/l)	Cu (mg/l)	SO4 (mg/l)
0	6.079	6.175	0	6.21	11.695	4979.74
1	5.982	3.475	1	6.011	8.23	4853.98
2	5.774	0.77	2	5.644	6.125	4882.55
4	5.537	0.125	4	5.512	5.505	4957.13
8	5.43	0.14	8	5.241	5.035	4789.4
12	2.894	0.115	12	5.361	4.96	4867.22
24	2.402	0.075	24	5.365	4.235	4989.4
36	1.876	0.07	36	5.205	4.672	5097.02
48	1.637	0.09	48	5.216	4.33	4987.8

Abiotic Matrixes Results

Zn+U+NaHCO3+Lactate+Na2S(250.16 mg/l)		Zn+U+NaHCO3+Lactate+Na2S(625.44 mg/l)	
Time (min)	Zinc (mg/l)	Time (min)	Zinc (mg/l)
0	11.6	0	10.88
5	0.48	5	5.22
10	0.78	10	5.44
15	0.7	15	6.12
20	2.68	20	6.88
25	3.34	25	7.1
30	2.02	30	7.22
40	2.68	40	6.64
50	2.18	50	4.3
60	1.2	60	5.1
90	0.96	90	2.9

Ni+U+NaHCO3+Lactate+Na2S(250.16 mg/l)		Ni+U+NaHCO3+Lactate+Na2S(625.44 mg/l)	
Time (min)	Nickel (mg/l)	Time (min)	Nickel (mg/l)
0	11.35	0	11.63
10	0.927	10	3.984
20	3.891	20	3.246
30	5.469	30	3.3
40	5.979	40	2.301
50	6.243	50	3.822
60	6.396	60	4.344
80	6.441	80	4.875
100	6.801	100	4.965
120	6.957	120	5.112
150	7.11	150	4.902

Uranium\ Sulfate\ Metal\ Ligand Matrixes Results

U+Zn+SO4+citrate			U+Ni+SO4+citrate			U+Citrate			
						Viable cell			Kill cell
Time (hr)	U (mg/l)	Zn (mg/l)	Time (hr)	U (mg/l)	Ni (mg/l)	Time (hr)	U(VI) (mg/l)	Total U (mg/l)	U (mg/l)
0	4.866	11.54	0	5.286	12.808	0	4.937	5.11	5.154
1	4.589	0.38	1	5.073	12.76	1	4.626	5.138	
2	4.56	0.2	2	5.303	12.74	2	4.585	5.288	
4	4.104	0.7	4	4.732	11	4	4.367	5.017	
8	3.874	0.48	8	4.224	1.66	8	3.43	5.253	
12	3.764	0.22	12	4.119	1.624	12	3.111	5.059	
24	3.685	0.4	24	3.841	1.356	24	2.977	5.287	
36	3.519	0.44	36	3.484	1.152	36	2.773	5.219	
48	3.361	0.44	48	3.256	0.352	48	2.624	5.094	
72	2.907	0.54	72	2.7	0.132	72	2.38	5.333	
96	2.398	0.34	96	2.667	0.1	96	1.854	4.987	4.979
120	2.233	0.24	120	2.593	0.0052	120	1.399	5.132	

U+Zn+SO4+EDTA				U+Ni+SO4+EDTA				U+EDTA		
								Viable cell		Kill cell
Time (hr)	U (mg/l)	Zn (mg/l)	SO4 (mg/l)	Time (hr)	U (mg/l)	Ni (mg/l)	SO4 (mg/l)	Time (hr)	U (mg/l)	U (mg/l)
0	6.6	12.78	6683.37	0	6.565	12.304	5228.6	0	5.934	6.355
1	6.1	12.52	6678.11	1	5.983	12.176	5116.83	1	5.992	
3.2	6.13	12.5	6545.31	2	6.237	11.8	4975.02	2	5.728	
4	6.01	12.38	6492.94	4	6.06	12.272	4930.02	4	6.216	
8	6.69	12.16	6460.83	8	5.952	11.368	4812.4	8	6.109	
12	5.77	12.1	6012.34	12	6.121	11.184	4735.7	12	6.185	
24	6.72	12.02	6117.59	24	6.269	11.808	4540.11	24	6.115	
36	6.16	12.12	5984.58	36	6.659	11.008	4499.4	36	6.231	
48	6.44	12.4	5943.51	48	6.197	11.824	4532.1	48	6.11	6.074

Standard Addition Results

addition (mg/l)	responds (mg/l)
-8.5	0
0	8.501
0.5	9.32
1	9.34
2	10.353
3	11.941

Y-12 Plant Wastewater Results

Time (hr)	U (mg/l)	Ni (mg/l)	Zn (mg/l)	Cu (mg/l)	SO4 (mg/l)
0	9.093	4.38	2.86	0.72	1417.41
1	8.965	4	2.02	0.34	1401.5
2	8.615	3.28	0.51	0.31	1293.51
4	8.075	3.22	0.08	0.3	1185.29
8	7.292	2.95	0	0.28	1010.32
12	6.898	2.8	0	0.29	990.3
24	5.693	2.76	0	0.28	918.18
36	4.72	2.69	0	0.28	909.98
48	4.665	2.63	0	0.26	913.53

Kill cell

TIMe	U	SO4	Zn	Ni	Cu
0	8.679	1461.51	2.688	4.476	0.61
12	8.771	1395.19	2.676	4.488	0.59
30	8.827	1459.37	2.704	4.412	0.58
48	8.749	1376.84	2.723	4.412	0.52

APPENDIX II

Calculation Results

Rate Constants of Heavy Metal Removal

Since the rates of U(VI) reduction and selected heavy metals removal followed a first order reaction in this research conditions, a first order kinetic expression (Snoeyink and Jenkins, 1980) was used to predict the rate constants in the study. According to this model, the rate of U(VI) reduction or seleted heavy metals removal can be expressed as:

$$\frac{d[C]}{dT} = -K[C]$$

where:

dC/dT = heavy metal removal rate of change in heavy metal concentration with time.

C = heavy metal concentration at any time, t.

K = rate constant.

A higher value of K indicated a higher rate of the heavy metal removal in the solution. Since test solutions were maintained under non-growth conditions, the cell concentration was assumed as constant.

Uranium Reduction Rate Constants
(Uranium only matrixes)

	K (hr^{-1})	R^2	σ
Low initial concentration	0.3113	0.9203	0.4750

Uranium Reduction Rate Constants
(uranium/sulfate matrixes)

		Different SO_4^{-2} Exp.A (10 mM lactate)		
I (m)	SO_4^{-2} (mg/l)	K1 (hr^{-1})	R^2	σ
	0	0.3113	0.9203	0.4750
0.0444	500	0.2132	0.9590	0.2284
0.0588	1000	0.0595	0.7296	0.5027
0.1684	5000	0.0254	0.9333	0.1251
0.2947	10000	0.0224	0.8222	0.1923

		Same I Exp. B		
I (m)	SO_4^{-2} (mg/l)	K1 (hr^{-1})	R^2	σ
0.2947	500	0.0476	0.9551	0.0961
0.2947	10000	0.0421	0.9577	0.0824

		Same Na^{+1} Conc. Exp.C		
I (m)	Na^{+1} (mg/l)	K1 (hr^{-1})	R^2	σ
0.1684	0.1349	0.0254	0.9333	0.1251
0.1328	0.1349	0.0805	0.8488	0.6266

		Different SO_4^{-2} (2 mM lactate) Exp. D		
I (m)	SO_4^{-2} (mg/l)	K1(hr^{-1})	R^2	σ
0.0444	500	0.0461	0.7847	0.4452
0.0588	1000	0.0655	0.6771	0.6279
0.1684	5000	0.0271	0.9885	0.0538

Uranium Reduction Rate Constants
(uranium/sulfate/metal matrixes)

Cu^{+2} (mg/l)	K1(hr^{-1})	R^2	σ
1	0.0627	0.6429	0.6483
3	0.0352	0.9386	0.1660
5	0.0300	0.9084	0.1757

Rate Constants
(uranium/sulfate/metal/organic ligand matrixes)

	Uranium reduction		
	K1(hr^{-1})	R^2	σ
Non-citrate	0.3113	0.9203	0.4750
Citrate	0.0091	0.9141	0.1195

	Uranium reduction			Zinc precipitation		
	K1(hr^{-1})	R^2	σ	K1(hr^{-1})	R^2	σ
Non-citrate	0.0325	0.8824	0.2185	> 1.0617	1.0	
Citrate	0.0059	0.9487	0.0588	> 0.8045	1.0	
	Uranium reduction			Nickel precipitation		
	K1(hr^{-1})	R^2	σ	K1(hr^{-1})	R^2	σ
Non-citrate	0.0398	0.8949	0.2512	0.1002	0.9972	0.0075
Citrate	0.0062	0.8823	0.0964	0.0568	0.9222	0.7050

Rate Constants
(Y-12 Plant wastewater)

	U^{+6} reduction			Zn^{+2} precipitation			Ni^{+2} Precipitation			
	K1 (hr^{-1})	R^2	σ	K1 (hr^{-1})	R^2	σ	K1 (hr^{-1})	K2 (hr^{-1})	R^2	σ
Synthetic	0.0595	0.7296	0.5027	>1.0617	1.0		0.1002		0.9972	0.0075
Y-12 Plant	0.0149	0.9483	0.0641	0.9391	0.9758	0.3097		0.0848	0.8568	0.4809

$dC/dT = K1 * C$ $dC/dT = K2 * C + A$

Statistical Analysis

A non-parametric statistical analysis 'Cox and Struat test' (Conover, 1980) was used to evaluate whether the U(VI) reduction trends were statistically lower in 2mM lactate matrixes than in 10 mM lactate matrixes. In this analysis, the U(VI) reduction percentages over time in the two matrixes were used as the comparison. The downward trends were detected at 5% ($p < 0.05$) level of significance. An example was shown as follows.

Example: (for 5000 mg/l of sulfate matrixes)

Time (hr)	D1(2mM lac.)	D2(10 mM lac.)	D1 %	D2 %
0	5.944	5.576	100	100
1	5.820	5.525	97.9	99.1
2	5.487	5.325	92.3	95.5
4	5.326	4.9	89.6	87.9
8	4.358	4.13	73.3	74.1
12	3.846	3.11	64.7	55.8
24	3.023	2.496	50.9	44.3
36	2.349	1.985	39.5	35.6
48	1.528	1.798	25.7	32.2

H_0: U(VI) reduction is not slower in 2 mM lactate solution than in 10 mM solution.

H_1: U(VI) reduction is slower in 2 mM lactate solution than in 10 mM solution.

Decision rule: n is the pair number, and p is 0.5. If $T \leq y$, or $T \geq n - y$, reject H_0.

Otherwise, accept H_0.

Solution:

Pair: (97.9, 99.1), (92.3, 95.5), (89.6, 87.9), (73.3, 74.1), (64.7, 55.8), (50.9, 44.3), (39.5, 35.6), (25.7, 32.2).

From the binomial distribution table (Conover, 1980), if $n = 9$, $T = (x1<x2) = 4$, $p = 0.5$, then $y = 2$. Since $T > y$, H_0 was accepted. Therefore, U(VI) reduction is not significant slower in 2 mM lactate solution than in 10 mM lactate solution.

Calculation of Sodium Sulfide Addition

Molecule weight: SO_4^{-2} (96), S^{-2} (32), $Na_2S \bullet 9H_2O$ (240.18).

1. For reducing 100 mg/l SO_4^{-2} :

   1). 100 mg/l of reduced SO_4^{-2} should yeald S^{-2} X1 mg/l.

$$SO_4^{-2} \rightarrow S^{-2}$$

$$X1 = \frac{100 \times 32}{96} = 33.33 \text{ mg/l}$$

   2). X2 mg/l $Na_2S \bullet 9H_2O$ is required to get 33.33 mg/l S^{-2} solution.

$$X2 = \frac{240.18 \times 33.33}{32} = 250.16 \text{ mg/l}$$

2. For reducing 250 mg/l SO_4^{-2} :

   1). 250 mg/l of reduced SO_4^{-2} should yeald S^{-2} X1 mg/l.

$$SO_4^{-2} \rightarrow S^{-2}$$

$$X1 = \frac{250 \times 32}{96} = 83.33 \text{ mg/l}$$

   2). X2 mg/l $Na_2S \bullet 9H_2O$ is required to get 83.33 mg/l S^{-2} solution.

$$X2 = \frac{240.18 \times 83.33}{32} = 625.44 \text{ mg/l}$$

VITA

Lingling Chu was born in Beijing, China. She graduated from Tsinghua University in Beijing with a Bachelors of Science degree in Environmental Engineering in 1982. After that, she worked, as an Environmental Engineer, in the Research Institute of Environmental Protection, Department of Chemical Industry, China. In August, 1993, she entered the Graduate School at the University of Tennessee at Knoxville. She worked as a graduate research assistant in the Civil and Environmental Engineering Department, while she pursued an Master of Science degree in Environmental Engineering. She received the degree in December, 1995.