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SAND96-1459 • UC-511

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Printed June 1996

Technical Considerations for the Implementation of Subsurface Microbial Barriers for Restoration of Groundwater at UMTRA Sites

Mark D. Tucker

Prepared by
Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550
for the United States Department of Energy
under Contract DE-AC04-94AL85000

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Technical Considerations for the Implementation of Subsurface Microbial Barriers for Restoration of Groundwater at UMTRA Sites

Mark D. Tucker
Senior Member of Technical Staff
Hanford Environmental Technology Department
Sandia National Laboratories
Albuquerque, NM 87185-0734

Abstract

The Uranium Mill Tailings Remediation Action (UMTRA) Program is responsible for the assessment and remedial action at the 24 former uranium mill tailings sites located in the United States. The surface remediation phase, which has primarily focused on containment and stabilization of the abandoned uranium mill tailings piles, is nearing completion. Attention has now turned to the groundwater restoration phase. One alternative under consideration for groundwater restoration at UMTRA sites is the use of in-situ permeable reactive subsurface barriers. In this type of a system, contaminated groundwater will be allowed to flow naturally through a barrier filled with material which will remove hazardous constituents from the water by physical, chemical or microbial processes while allowing passage of the pore water. The subject of this report is a reactive barrier which would remove uranium and other contaminants of concern from groundwater by microbial action (i.e., a microbial barrier). The purpose of this report is to assess the current state of this technology and to determine issues that must be addressed in order to use this technology at UMTRA sites. The report focuses on six contaminants of concern at UMTRA sites including uranium, arsenic, selenium, molybdenum, cadmium and chromium. In the first section of this report, the fundamental chemical and biological processes that must occur in a microbial barrier to control the migration of contaminants are described. The second section contains a literature review of research which has been conducted on the use of microorganisms to immobilize heavy metals. The third section addresses areas which need further development before a microbial barrier can be implemented at an UMTRA site.

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Section 1

Introduction

The Uranium Mill Tailings Remediation Action (UMTRA) Project was authorized by the Uranium Mill Tailings Radiation Control Act of 1978 (UMTRCA). This act established a program of assessment and remedial action at 24 inactive uranium mill sites in the United States to dispose of, stabilize, and control residual radioactive material. Residual radioactive material is defined as "radioactive waste in the form of tailings resulting from the processing of ores for the extraction of uranium and other valuable constituents, and other radioactive waste at a processing site which relates to such processing" (DOE/UMTRA, 1993). The purpose of the UMTRA project is to ensure that radiological and nonradiological hazards at the sites do not exceed the standards established by the EPA for the protection of public health, safety, and the environment.

Uranium mining was an important economic activity in the United States for many years following World War II. However, due to the declining interest in nuclear power and the availability of inexpensive uranium from foreign sources, this activity has diminished in recent years and most uranium mines have been abandoned. In most cases, the mill tailings piles associated with the mineral processing plants near those mines have been left in place or relocated in nearby engineered landfills. It is estimated that more than 230 million tons of uranium mill tailings are present at former mill sites throughout the United States (Morrison and Spangler, 1992). These mill tailings piles contain low concentrations of uranium and other contaminants which were not economically recoverable during mining operations (Thomson and Heggen, 1983).

Previously, most work in the UMTRA project has centered on surface activities to contain and stabilize uranium mill tailings piles. Because the surface remediation phase has a specific termination date and is nearing completion, attention has now turned to groundwater remediation. The groundwater restoration phase began in 1991 and currently has no specific time limitations. Tailings-related contamination of groundwater is present beneath and down-gradient from many of the processing sites. Regulated constituents in groundwater whose concentrations or activities exceed maximum concentration limits (MCL) or background levels at one or more sites include, but are not limited to, uranium, selenium, arsenic, molybdenum, chromium, cadmium, sulfate, nitrate, gross alpha, radium-226 and radium-228 (DOE/UMTRA, 1991a). It has been estimated that the remediation of groundwater at the 24 UMTRA sites using conventional approaches will cost at least one billion dollars (Morrison and Spangler, 1992).

Pump and treat systems have been the conventional approach to groundwater remediation. However, it has been estimated that groundwater remediation at large, geologically complex sites similar to those found in the UMTRA program will take decades to complete using pump and treat systems. In addition, the effectiveness of pump and treat methods to remediate contaminated groundwater to drinking water standards has been questioned (Hoffman, 1992). As a result of these concerns, several alternatives to conventional pump and treat systems are being considered including enhanced pump and treat systems, electrokinetics, bioremediation, impermeable subsurface barriers, and permeable reactive subsurface barriers.

The use of in-situ permeable reactive subsurface barriers is a promising alternative for some groundwater systems. In this type of a system, contaminated groundwater will be allowed to flow naturally through a barrier filled with material which will remove the hazardous constituents from the water by physical, chemical or microbial processes. A schematic of an in-situ reactive permeable barrier is illustrated in Figure 1 at the end of this section.

Contaminant removal in a subsurface permeable barrier by microbial action is the subject of this report. In a microbial barrier, a population of microorganisms would be maintained to retard the migration of heavy metal and radionuclide contaminants in the groundwater. The microbial population within the barrier would consist of a mixed culture of microorganisms, including sulfate-reducing bacteria, which would utilize the high sulfate concentrations found in groundwater at most UMTRA sites (DOE/UMTRA, 1991b). Sulfate-reducing bacteria are important because they have been shown to mediate reactions that cause the chemical reduction of uranium and other heavy metals (Lovley et al., 1991; Lovley and Phillips, 1992a; Lovley and Phillips, 1992b). By encouraging the growth of sulfate-reducing bacteria and other microorganisms in the barrier, heavy metals and radionuclides may be deposited within the barrier matrix material since most of these contaminants are insoluble in their reduced state. After a period of time had elapsed, the barrier material (along with the precipitated metal contaminants) could be excavated and disposed of in an approved manner, possibly with the recovery of the precipitated metals. Alternatively, if the precipitated phases of the heavy metal and radionuclide contaminants are shown to be stable, the barrier material could be left in place.

This report focuses on the use of a microbial barrier to remove contaminants from UMTRA groundwaters. Six contaminants of concern will be considered in this report including uranium, arsenic, selenium, molybdenum, cadmium, and chromium. Each of these contaminants have been detected at levels above drinking water standards in the groundwater at one or more UMTRA sites (DOE/UMTRA, 1991a). This report contains

three major sections. In the first section, the fundamental chemical and biological processes that must occur in the microbial barrier to remove U, As, Se, Mo, Cd, and Cr from groundwater are described. The second section contains a literature review of current research that will be important in the development of a microbial barrier for UMTRA sites. Research concerning immobilization of heavy metals via microbial activity and implementation of permeable reactive subsurface barriers are covered in the literature review. The final section of this report identifies areas where further development is required in order to implement a microbial barrier at UMTRA sites.

Figure 1: In-situ Permeable Reactive Subsurface Barrier for Groundwater Remediation (Thomson et al., 1991)

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Section 2

Chemical and Biological Processes for Contaminant Removal

The purpose of this report is to address technical considerations concerning the use of microbial barriers to remove heavy metals and radionuclides from UMTRA groundwaters. Six contaminants of concern will be considered including U, As, Se, Mo, Cd, and Cr. Unlike organic compounds, heavy metals and radionuclides cannot be destroyed or mineralized by microorganisms during the remediation process. Instead, heavy metals must be removed from water by chemical, physical or biological processes such as adsorption, precipitation, ion exchange or oxidation/reduction reactions. Certain specialized microorganisms have the ability to alter the solubility of heavy metals and radionuclides and to cause these contaminants to precipitate from solution. These microorganisms may be utilized in a microbial barrier to cause heavy metal and radionuclides to be immobilized in the barrier matrix material.

Until recently, most research on microbe-metal interactions has focused on aerobic bacteria. Aerobic bacteria gain their energy for maintenance and growth by coupling the oxidation of organic compounds (or other reduced compounds such as H_2 or NH_4) to the reduction of O_2 . Aerobic organisms also have highly developed systems to handle metallic elements including systems to sequester metals from the environment for the synthesis of metal-containing enzymes and co-factors. A few aerobic bacteria, known as chemoautotrophic bacteria, may play a role in immobilizing metals in the environment by oxidizing reduced forms of iron and manganese to metal oxides. However, more environmentally significant metal reactions take place under anaerobic conditions. Under these conditions, which are prevalent in many subsurface environments, metal-reducing microorganisms use metals in a manner which is analogous to O_2 utilization by aerobic organisms. This process is called dissimilatory metal reduction and occurs when electrons from organic compounds, H_2 , or inorganic compounds such as elemental sulfur are transferred to oxidized forms of metals such as iron, manganese, uranium, or selenium. Electron transport to oxidized metals during dissimilatory metal reduction can provide energy for growth just as electron transport to O_2 provides energy for growth in aerobic bacteria. This process is referred to as dissimilatory metal reduction to distinguish it from assimilatory metal reduction which is the process used by aerobic bacteria to assimilate metals into cellular components (Lovley, 1993).

Most research on dissimilatory metal reduction has been focused on sulfate-reducing bacteria. Sulfate-reducing bacteria are chemoheterotrophic, mesophilic, strictly anaerobic bacteria that occur naturally in soil systems. Some of the most abundant sulfate-reducing bacteria in many soil systems are *Desulfovibrio desulfuricans*. Most members of the *Desulfovibrio* genus of bacteria have a curved shape and are fairly easy to isolate and purify (Postgate, 1979). The mesophilic *Desulfovibrios* have an upper temperature limit of 45 to 48°C and their best growth occurs around 30°C. Optimum pH for growth is between 5.5 and 8.5. However, they are known to grow in acidic environments well below their optimum pH. It is thought that they grow in microniches where the pH is higher than the overall system pH (Zehnder, 1988). Most sulfate-reducing bacteria grow on low molecular weight organic acids such as lactate, pyruvate, or acetate. However, some have been known to grow with H₂ as the energy source while fixing CO₂ as the carbon source by utilizing the Calvin cycle (Zehnder, 1988).

Growth of sulfate-reducing bacteria and other microorganisms within the microbial barrier could initiate the removal of metals from groundwater by three different mechanisms. The first mechanism is dissimilatory metal reduction as discussed above. This process is also referred to as anaerobic respiration. Microbial reduction of uranium by anaerobic respiration has been studied extensively (Lovley et al., 1991; Lovley and Phillips, 1992a; Lovley and Phillips, 1992b).

Uranium usually exists in either the U(VI) or U(IV) oxidation state. U(VI), which occurs under oxidizing conditions, is soluble in water and, as a result, is very mobile in the environment. U(VI) also forms aqueous carbonate complexes [i.e., UO₂CO₃⁰, UO₂(CO₃)₂²⁻ and UO₂(CO₃)₃⁴⁻] which increases its solubility in natural water. Under reducing conditions uranium exists as U(IV) which is generally insoluble in water as the UO_{2(s)} (uraninite) phase or USiO₄ (coffinite) phase (Brookins, 1988). Therefore, U(IV) is not usually subject to aqueous transport and is fairly immobile in the environment. So the fundamental process that must occur in the barrier to immobilize uranium is the reduction of U(VI) to U(IV). Aqueous chemistry of uranium is summarized in the Eh-pH diagram for the U-C-O-H system which is shown in Figure 2 at the end of this section.

Recent studies which have been cited above have shown that certain anaerobic microorganisms, such as the sulfate-reducing bacteria *Desulfovibrio desulfuricans*, can mediate the reduction of U(VI) to U(IV) according to the following reaction.

In this reaction, low molecular weight organic compounds, such as lactate, acetate or pyruvate serve as the substrate (electron donor and energy source) and uranium serves as the terminal electron acceptor. Uranium forms the insoluble uraninite compound and is precipitated from solution.

Other metals, in addition to uranium, can also serve as electron acceptors during microbial mediated reactions. Microbial reduction of selenium has also been demonstrated (Maiers et al., 1988). During microbial reduction, soluble oxidized selenium (SeO_4^{2-} or SeO_3^{2-}) is reduced to insoluble elemental selenium (Se^0). Microorganisms that can mediate the selenium reduction reaction include *Wolinella succinogenes*, *Desulfovibrio desulfuricans* and *Pseudomonas* bacteria (Macy et al., 1989; Tomei et al., 1992; Tomei et al., 1995). The chemistry of selenium is closely related to sulfur because of the similarity of their electron structure which is demonstrated by the fact that they are in the same column in the periodic chart. Like S, Se has four oxidation states. Under oxidizing conditions, Se forms soluble oxyanions (SeO_4^{2-} or SeO_3^{2-}). Under reducing conditions, Se forms the selenides (H_2Se , and HSe^-) both of which form insoluble precipitates with common cations such as Fe, Cu, and Pb (Thomson, 1989). Oxidation and reduction of Se is thought to be microbially mediated (Maiers et al., 1988). Therefore, in a barrier, selenium could be reduced from Se(VI) to Se(-II) or elemental Se by microbial action and precipitated from solution. The aqueous chemistry of selenium is summarized in the Eh-pH diagram shown in Figure 3.

A second mechanism initiated by sulfate-reducing bacteria that could occur to remove metals from groundwater is sulfide precipitation. Many metals, such as Mo, Cd, As, Cu, Zn, Pb, Fe, and Mn precipitate as metal sulfides (Hammack et al., 1994; Brookins, 1988). Growth of sulfate-reducing bacteria generates sulfide according to the following reaction.

The increased sulfide concentration in water could cause the precipitation of molybdenum, cadmium, and arsenic according to the following reactions.

Since the solubility of MoS_2 , CdS and As_2S_3 is extremely low, these contaminants and other metals that precipitate as sulfides may be effectively removed from the groundwater by the production of biogenic sulfide.

Molybdenum species in natural waters under oxidizing conditions is dominated by Mo(V) and Mo(VI) ions (i.e., HMoO_4^- , MoO_4^{2-} , MoO_2^+). Under oxidizing conditions, molybdenum is transported in a similar manner as oxyanions of other contaminants such as U, Se, As, and V. Under strongly reducing conditions, molybdenum is reduced to insoluble Mo(IV) compounds such as molybdenite (MoS_2). Because the chemistry of molybdenum is similar to uranium, this element is often found in conjunction with uranium deposits. Some molybdenum is separated during the mill process and ends up in tailings piles. Molybdenum is soluble under high Eh conditions and can be easily transported in the presence of water. Since it is also known toxin, the leachate from mill tailings piles must be monitored for molybdenum. To date, dissimilatory metal reduction of soluble Mo(VI) to insoluble Mo(IV) has not been demonstrated. However, some microorganisms have been shown to have the ability to reduce Mo(VI) to Mo(V) which is also soluble (Sugio et al., 1988; Cambell et al., 1985). In order to remove molybdenum from groundwater in a microbial barrier, Mo(VI) must be reduced to Mo(IV) which can then be precipitated in the presence of biogenic sulfide as $\text{MoS}_{2(s)}$. The effect of Eh and pH on Mo solubility for the Mo-S-O-H system is summarized in Figure 4 (Brookins, 1988).

Cadmium is a serious environmental pollutant due to its high toxicity. However, unlike many other transition metals, cadmium only displays the +2 oxidation state in natural waters. Under oxidizing conditions, cadmium solubility is controlled by carbonates such as $\text{CdCO}_{3(s)}$ (otavite) or hydroxides such as $\text{Cd(OH)}_{2(s)}$. Under reducing conditions, cadmium solubility is controlled by the sulfide phase CdS . The carbonate and hydroxide phases of cadmium are several orders of magnitude more soluble than the sulfide phase. Therefore, Cd will only be mobile if the pH is low and strongly reducing conditions (represented by the presence of biogenic sulfide) are not present in solution (Thomson, 1989). The mechanism for cadmium removal in a microbial barrier will be precipitation of CdS . Cadmium chemistry is summarized in a pH-Eh diagram in Figure 5 (Brookins, 1988).

Arsenic behaves in a manner similar to sulfur and selenium in that it also has four oxidation states. Arsenic is soluble in its oxidized form as arsenates (AsO_4^{3-}) and arsenites (AsO_3^{3-}). Under reducing conditions, insoluble precipitates such as $\text{As}_2\text{O}_{3(s)}$ (arsenolite) or the sulfides $\text{AsS}_{2(s)}$ and $\text{As}_2\text{S}_{3(s)}$ are formed. Arsenic also undergoes methylation producing organic species such as $\text{HAsO}_2(\text{CH}_3)_2$ (dimethyl arsinic acid), $\text{H}_2\text{AsO}_3\text{CH}_3$ (methylarsonic acid), or $\text{HAs}(\text{CH}_3)_2$ (dimethyl arsine) which enhances its

solubility (Thomson, 1989). The solubility of As is also controlled by iron species. It is thought that arsenic is sorbed onto the surfaces of ferric (Fe^{3+}) iron precipitates which occur under oxidizing conditions. As a solution becomes reducing, Fe^{3+} is reduced to soluble Fe^{2+} species desorbing arsenic and releasing it into solution (Masscheleyn et al., 1991). Therefore, in some cases, the concentration of arsenic may increase as the solution changes from oxidizing to more reducing conditions. Although the chemistry of arsenic is more complex than the elements that have been previously discussed, the mechanism for removal of arsenic in a microbial barrier will most likely be the reduction of As(V) to As(III) and precipitation from solution. The chemistry of arsenic is summarized in the Eh-pH diagram shown in Figure 6.

There are metal contaminants of concern at UMTRA sites that will not precipitate as sulfides and will not serve as terminal electron acceptors during anaerobic respiration. However, some of these metals can be removed from solution by oxidation-reduction reactions. These redox reactions can be initiated by the strongly reducing conditions that result from the generation of sulfide by sulfate-reducing bacteria. This is a third mechanism that may act to remove metal contaminants from groundwater in the microbial barrier. Chromium, for example, exists in two oxidation states in natural water systems. Under oxidizing conditions, Cr(VI) is soluble as chromate (CrO_4^{2-}). Under reducing conditions, Cr(III) is generally insoluble in water as $\text{Cr}_2\text{O}_3(\text{s})$ (eskolaite) (Thomson, 1989). Cr(VI) is considered to be many times more toxic than Cr(III). In the permeable barrier, the following reaction could occur to reduce chromium from Cr(VI) to Cr(III) (Fude et al., 1994).

The effects of reduction on Cr solubility are shown in the Eh-pH diagram in Figure 7.

In summary, it is believed that all six contaminants of concern addressed in this report (U, As, Se, Mo, Cd, and Cr) can potentially be removed from groundwater at UMTRA sites by a microbial barrier. Removal of U and Se from water by dissimilatory metal reduction has been demonstrated in the laboratory. Removal of Mo, Cd, and As from water by sulfide precipitation is well known. Chromium can be precipitated from water by chemical reduction due to strongly reducing conditions caused by the generation of biogenic sulfide. However, precipitation of Mo by biogenic sulfide requires the reduction of Mo(VI) to Mo(IV). Reduction of Mo by microbial action has not been demonstrated in the laboratory.

Figure 2: Eh-pH Diagram for the U-C-O-H System, Total Concentration of $U = 10^{-6} \text{ M}$, $C = 10^{-3} \text{ M}$ (Brookins, 1988)

Figure 3: Eh-pH Diagram for Se-O-H System, Total Concentration of Se = 10^{-6} M (Brookins, 1988)

Figure 4: Eh-pH Diagram for Mo-S-O-H System, Total Concentration of $Mo = 10^{-8} \text{ M}$, $S = 10^{-3} \text{ M}$ (Brookins, 1988)

Figure 5: Eh-pH Diagram for Cd-C-S-O-H System, Total Concentration of Cd = 10^{-8} M, C = 10^{-3} M, S = 10^{-3} M (Brookins, 1988)

Figure 6: Eh-pH Diagram for As-S-O-H System, Total Concentration of As = 10^{-6} M, S = 10^{-3} M (Brookins, 1988)

Figure 7: Eh-pH Diagram for Cr-O-H System, Total Concentration of Cr = 10^{-6} M (Brookins, 1988)

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Section 3

Literature Review

The ability of microbes to use compounds other than oxygen as the terminal electron acceptor in respiration under anaerobic conditions has been known for many years. However, the ability of anaerobic microbes to reduce certain metals, such as iron and manganese, has only recently been discovered. Much of the previous research in this area has been conducted by Derek R. Lovley and his collaborators at the U.S. Geological Survey in Reston, Virginia. In the late 1980s, Lovley and his co-workers isolated an Fe(III) and Mn(IV)-reducing microorganism from freshwater sediments of the Potomac River and designated it GS-15. In a subsequent series of studies, they grew GS-15 in an anaerobic medium with acetate, ethanol, butyrate, or propionate as the sole electron donor and Fe(III), Mn(IV), or nitrate as the sole electron acceptor (Lovley and Phillips, 1988). Fe(III) was optimally reduced to Fe(II) at pH 6.7 to 7 and at 30 to 35°C. Mn(IV) was reduced to Mn(II) and nitrate was reduced to ammonia. This study was the first demonstration that microorganisms can completely oxidize organic compounds with Fe(III) or Mn(IV) as the sole electron acceptor. In another study, Lovley and his co-workers grew GS-15 in an anaerobic medium with toluene as the sole electron donor and an Fe(III) oxide as the electron acceptor and found that growth of the microorganism coincided with Fe(III) reduction (Lovley and Lonergan, 1990). Toluene was completely oxidized to CO₂ and magnetite (Fe₃O₄) was the iron end product. GS-15 was the first microorganism known to couple the oxidation of aromatic compounds to the reduction of Fe(III).

Subsequently, Lovley and his co-workers turned their attention to uranium and began to investigate the ability of the GS-15 microbe (now called *Geobacter metallireducens*) and other microorganisms to reduce U(VI) to U(IV). In a 1991 study, they found that microbial reduction of U(VI) by GS-15 was much faster than commonly cited abiological mechanisms for U(VI) reduction (Lovley et al., 1991). In 1992, they used *Shewanella putrefaciens*, another iron-reducing microbe, and *Desulfovibrio desulfuricans*, a sulfate-reducing bacteria, to reduce uranium (Lovley and Phillips, 1992a; Lovley and Phillips, 1992b). In the latter experiments, sulfate and U(VI) were reduced simultaneously and resulted in the extracellular precipitation of the U(IV) mineral uraninite (UO₂). Enzymatic reduction of U(VI) by *Desulfovibrio desulfuricans* was much faster than nonenzymatic reduction of U(VI) by sulfide, even when dead cells of bacteria were added to provide a potential catalytic surface for the nonenzymatic reaction. In addition, *Desulfovibrio*

desulfuricans reduced U(VI) faster than GS-15. During the experiments, the concentration of uranium in aqueous streams was decreased from initial concentrations as high as 24 mM to final concentrations below 0.1 mM.

Earlier work in this area was also performed by Kauffman and others at Kerr-McGee Corporation in Oklahoma City, Oklahoma (Kauffman et al., 1986). They used bacteria belonging to the genus *Clostridium* and *Desulfovibrio* to reduce the concentration of uranium, selenium, molybdenum and sulfate in mine water. However, Kauffman and his co-workers concluded that uranium was not reduced from U(VI) to U(IV) by a biological process. Instead, they believed that hydrogen sulfide (H_2S) produced by the sulfate-reducing bacteria reduced U(VI) by a chemical reaction.

Other workers have also investigated the microbial transformations of various metals. Dvorak et al. (1992) used anaerobic reactors to treat metal-contaminated water. Concentrations of aluminum, cadmium, manganese, iron, nickel and zinc were decreased by over 95% in the reactors. Silver (1987) found that iron-oxidizing bacteria in mill tailings piles are responsible for the oxidation of pyrite (FeS_2) which results in the generation of sulfuric acid (H_2SO_4). Sulfuric acid reacts with minerals such as uranium, thorium and radium in the mill tailings piles to cause the solubilization of the heavy metals and radioactive nuclides. The migration of those substances into groundwater and surface water can cause the deterioration of those water systems.

Additional information concerning sulfate-reducing bacteria was provided by other workers. Middleton and Lawrence (1977) calculated kinetic coefficients for microbial sulfate reduction by a mixed culture of sulfate-reducing bacteria which utilized acetic acid as the substrate. They found that the Monod equation can be used to model the growth of bacteria in the system and that the effect of temperature on the system can be modeled using an Arrhenius type relationship. Thomson (1987) used strongly reducing conditions produced by sulfate-reducing bacteria to precipitate various metals and metalloids (copper, chromium and selenium) from an aqueous stream. Thomson (1987) also conducted studies on the growth kinetics of a mixed culture of sulfate-reducing bacteria and determined that only chromium inhibited bacterial growth in the bioreactor. For every case that was studied, the system was able to achieve greater than 90% removal of the metals. Magee et al. (1978) conducted kinetic studies on *Desulfovibrio vulgaris* and *Desulfovibrio gigas* bacteria. They found that efficiency of growth varied with electron donor-acceptor combinations and that differences in energy coupling occurred with the various bacterial strains.

Tomei et al. (1992) adapted cultures of *Wolinella succinogenes* to grow in the presence of 1 mM SeO_3^{2-} or 10 mM SeO_4^{2-} which were reduced to red, amorphous,

elemental selenium after the culture reached the stationary growth phase. Bacterial cells taken from an active, selenium-reducing culture were examined by transmission electron microscopy and found to have large, electron-dense granules in the cytoplasm which consisted of selenium. *W. succinogenes* was unable to grow with SeO_3^{2-} or SeO_4^{2-} as the final electron acceptor. Tomei et al. (1995) investigated the transformation of selenate and selenite to elemental selenium by *Desulfovibrio desulfuricans*. They found that this bacteria can be adapted to grow in the presence of 10 mM selenate and 0.1 mM selenite. Electron microscopy with energy-dispersive X-ray diffraction analysis showed that selenate and selenite were reduced to elemental selenium which accumulated outside of the cells. Growth did not occur with either selenate or selenite as the electron acceptor.

Reduction of Cr(VI) by a consortium of sulfate-reducing bacteria was investigated by Fude et al. (1994). The consortium consisted of a range of bacteria that had the ability to reduce Cr(VI) to Cr(III) as amorphous precipitates which were associated with the bacterial surfaces. The consortium included sulfate-reducing bacteria. Approximately 80-95% of Cr was removed from solutions that began with concentrations between 50 to 2000 ppm Cr. Gamma-irradiated, killed cells did not remove the metal. Cr removal was not inhibited by the addition of U or Zn to the media which were also removed by incorporation into the Cr precipitate. The consortium was tolerant to small amounts of oxygen in the headspace of the test vessels, but active growth of the bacteria was necessary for Cr removal. These researchers concluded that Cr(VI) was reduced by an indirect mechanism [i.e., the bacteria produced H_2S which acted as a reducing agent for Cr(VI)].

Shen and Wang (1994) also used *Escherichia coli* to reduce Cr(VI) to Cr(III). Cr(VI) was reduced under both aerobic and anaerobic conditions by using a variety of electron donors including glucose, acetate, propionate, glycerol, and glycine. Cr(VI) reduction occurred faster under anaerobic conditions than under aerobic conditions most likely due to the fact that, under aerobic conditions, oxygen is a strong competitor of electrons. Several heavy metals and phenolic compounds, including Cu^{2+} and Zn^{2+} , were toxic to Cr(VI) reduction. It was concluded that redox potential alone was not an important factor in Cr(VI) reduction by *E. coli*. Cell growth was not required for Cr(VI) reduction since resting cells were also found to have the capability to mediate this reaction. Chromium removal did not occur in experiments conducted with heat killed cells indicating that the mechanism for chromium removal was not bioadsorption. A good fit of the experimental data to the Arrhenius equation confirmed that biochemical reactions and not absorption were involved in Cr(VI) reduction. Conclusions from this research were that the mechanism of Cr(VI) reduction appeared to be enzymatic, since it was observed

that the reaction was catalyzed by cell extracts and was not affected by the redox potential of the culture media.

Masscheleyn et al. (1991) studied the effect of redox potential and pH on arsenic speciation and solubility in contaminated soil. Alterations in the oxidation state of arsenic greatly influenced its solubility. At high redox values (500-200 mV), arsenic existed mainly as As(V) and its solubility was low. An alkaline pH, or reduction of As(V) to As(III) increased its solubility. Under moderately reducing conditions (0-100 mV), arsenic solubility was controlled by the dissolution of iron hydroxyoxides. Upon reduction to -200 mV, arsenic was even more soluble (approximately 13-fold more soluble than at 500 mV). Under reducing conditions, arsenic solubility was controlled by a $\text{Mn}_3(\text{AsO}_4)_2$ phase.

Seyler and Martin (1989) studied biogeochemical processes affecting arsenic speciation in a permanently stratified lake. Thermodynamic calculations lead to the prediction that, at equilibrium, As(V) (as arsenate) should be the only stable oxidation state under oxic conditions and As(III) (as arsenite) should be the only stable oxidation state under anoxic conditions. However, the arsenate and arsenite concentrations versus depth did not reflect the expected thermodynamic equilibria, which indicated a slow and incomplete response to the redox conditions. Several biotransformations involving bacteria, fungi, and algae have been observed in the laboratory that could explain this disequilibrium. This research indicated that the reduction of As(V) to As(III) is not purely a chemical process but that the reduction must be mediated by microbial catalysts.

Much less research has been conducted on microbially mediated molybdenum reduction. As already stated above, some early research was performed by Kauffman et al. (1986). These workers found that percolation of uranium mine discharge water through Ambrosia Lake, NM soil was effective in lowering the selenium, uranium, molybdenum, and sulfate concentrations in the water. They concluded that sulfate-reducing bacteria in the soil metabolize sulfate to sulfide which reacts with molybdate (MoO_4^{2-}) ions to form insoluble molybdenum species. However, the exact mechanism for Mo removal was not thoroughly investigated during the course of their research to definitely conclude that Mo reduction was a chemical reaction instead of a microbially mediated reaction. Sugio et al. (1988) found that, in the presence of phosphate ions, Mo^{6+} was reduced enzymatically by washed intact cells of *Thiobacillus ferrooxidans* to give molybdenum blue. Molybdenum blue is a complex compound where the mean oxidation state of Mo is thought to be between 5 and 6 [$\text{MoO}_{2.0}(\text{OH})$ and $\text{MoO}_{2.5}(\text{OH})_{0.5}$]. The enzyme responsible for Mo reduction was determined to be sulfur:ferric ion oxidoreductase (SFORase). This reaction occurred under acidic

conditions and elemental sulfur was oxidized to sulfite to provide an energy source. The process was completely inhibited by Fe^{3+} , Cu^{2+} , and Co^{2+} . Laboratory studies were conducted to determine the solubility of Mo in evaporation pond soils under varying redox conditions by Amrhein et al. (1993). These researchers found that reduction and precipitation of Mo, apparently as MoS_2 , occurred under reducing conditions but that Mo was resolubilized in less than one day when the soil sample was reaerated.

Also included in the literature review were several books and papers that cover the general topic of heavy metal solubility. Brookins (1988) calculated Eh-pH diagrams for numerous elements. The Eh-pH diagrams for U, As, Se, Mo, Cd, and Cr are included in this report. Anderson (1987) found that uranium was not reduced chemically by hydrogen sulfide in the deep seawater Cariaco Trench despite the anoxic conditions that exist in the basin. This suggests that U(VI) is not chemically reduced to insoluble U(IV) in marine systems, even in the presence of hydrogen sulfide. Thomson and Heggen (1983) provide an overview of uranium mining and milling activities in the western U.S. along with a discussion on uranium geochemistry.

Some fundamental work has been done on microbial barriers. However, most of the work has centered on the use of microbial barriers to remove organic compounds from groundwater. Gillham and Burris (1992) provide an excellent review of all types of permeable subsurface reactive barriers. In the section on microbial barriers, they point out that development of these barriers has come about because of the limited ability to mix contaminated water, oxygen and nutrients in traditional bioremediation methods. Kao and Borden (1992) proposed the use of a two-layer permeable reactive barrier to remove BTEX from groundwater. The up-gradient layer of the wall would be composed of concrete briquets laden with nutrients while the down-gradient layer would contain peat. Laboratory column studies using this concept over a period of 55 days showed rapid degradation of toluene and ethylbenzene, lower degradation rates for xylene and almost no degradation of benzene. Thomson et al. (1992) describe the results of laboratory column studies where removal efficiencies for BTEX and methyl tertiary-butyl ether (MTBE) of 95% were observed. These compounds were removed from a combination of air sparging and biodegradation.

Taylor et al. (1993) conducted research on the use of a resting-cell (i.e., non-dividing) methanotrophic microbial filter (biofilter) for remediating migrating subsurface plumes of trichloroethylene (TCE). In this system, methanotrophic microorganisms (*Methylosinus trichosporium*) were injected into a test bed ahead of an migrating TCE plume. These bacteria have the capability to attach to soil or rock and form a zone of enhanced biodegradative activity and contain the enzyme methane monooxygenase (MMO) which

has been shown to degrade TCE by co-metabolism. After bacterial injection into the test bed, a biofilter approximately 0.1 m thick was established in the flow direction. Following the development of the biofilter, a 24 hour pulse of TCE at a concentration of 109 ppb was pumped into the test bed at the inlet port. No TCE was detected immediately down-gradient of the filter (the detection limit was 0.5 ppb). The permeability of the test bed was measured before bacterial inoculation and after completion of the test series. No measurable change in permeability was observed, confirming the absence of biofouling. The authors concluded that the results demonstrate that a biofilter was established by injecting resting bacterial cells and that this biofilter removed TCE down to the detectable limit.

There has been some research on the use of microbial barriers to remove inorganic compounds. Robertson and Cherry (1995) used finely ground sawdust as a substrate in a microbial barrier designed to denitrify septic tank effluent in a drainfield under anaerobic conditions. Nitrate levels in the barrier were decreased from 125 mg/l NO_3^- -N to as low as 1.2 mg/l. However, even more important as far as the use of microbial barriers at UMTRA sites is concerned, they found that significant sulfate reduction also occurred which would seem to indicate that sulfate-reducing bacteria were also able to grow in the barrier. Presumably, fermentation products from the mixed culture of bacteria within the barrier supply the indigenous sulfate-reducers with the required low molecular weight organic acids for substrate.

Miller et al. (1987) provides a detailed look at the types of microorganisms typically found at uranium mill tailings sites. Samples were collected in and around the mill tailings at Edgemont, South Dakota, and Rifle and Maybell, Colorado. *Arthrobacter* were found to be the predominant microorganism inhabiting the sandy tailings while *Bacillus* and fungi predominated the slime tailings. Sulfate-reducing bacteria were isolated in low numbers from tailings samples but were isolated in higher numbers from topsoil in contact with the tailings. The authors conclude that uranium mill tailings sites are capable of supporting a wide variety of microbial populations and that these microorganisms may play a significant role in the mobilization or immobilization of contaminants at these sites.

Section 4

Development Needs

A. Introduction

The microbial barrier is a promising concept for groundwater remediation. Advantages of the use of a microbial barrier over barriers that contain other reactive material for removal of heavy metals and radionuclides are:

1. Microbial barriers use natural processes to remove heavy metals and radionuclides from groundwater. In many cases, these processes emulate the natural processes that caused the original deposition of metal ores. For example, the deposition of uranium ores [i.e., $\text{UO}_2(\text{s})$ or $\text{USiO}_4(\text{s})$] is thought to occur as a result of microbial processes.
2. Microorganisms have the ability to maintain strongly reducing conditions in a barrier which would cause the precipitation of many contaminants of concern. For example, the reduction and removal of chromium occurs as a result of strongly reducing conditions.
3. When high sulfate concentrations occur in groundwater, microorganisms in a barrier would naturally produce H_2S , a strong reducing agent which forms insoluble precipitates with many heavy metals. For example, Mo, As, and Cd all form insoluble sulfide precipitates.
4. Microbial enzymes catalyze many reactions making them faster or more complete. For example, reduction of U(VI) to U(IV) by *Desulfovibrio desulfuricans* occurs in a matter of hours but the same reaction with H_2S takes several days. This would reduce the required residence time in a barrier resulting in a smaller, less costly design.

Disadvantages to a microbial barrier as compared to the use of other reactive material in a barrier include the following:

1. The proper conditions (i.e., temperature, Eh, pH, etc.) must be maintained in a barrier to sustain growth of microorganisms.
2. A period of time will be required to establish the microorganisms in a barrier. Other reactive material may be effective immediately upon installation. This may

especially be a problem if the barrier material needs to be periodically excavated and replaced.

3. Monitoring of a barrier may be more expensive since microbiological analyses may be required.
4. The chemistry is far more complex than other types of barriers.

Despite the fact that a microbial barrier is a promising concept for an alternative to traditional groundwater remediation methods, this technology is still in its infancy. Several important issues remain to be resolved before a microbial barrier could be implemented in the field. Some of these issues include:

1. Understanding of the fundamental processes of contaminant removal in the barrier, especially for contaminants other than uranium.
2. Determination of methods to supply organic substrate, nutrients, and electron acceptors for sustained growth of microorganisms in the barrier.
3. Development of methods for the physical emplacement of the barrier in the proper location at the required depths and methods to configure the reactive bed to attain the required permeability.
4. Development of models to determine the effective capture zone of the barrier.
5. Understanding of the effects of transient changes in groundwater chemistry on contaminant immobilization in the barrier.
6. Understanding of the need for or timing of replacing of the reactive bed in the barrier.
7. Development of systems to monitor the performance of the barrier.

The remainder of this report addresses these key issues in greater detail.

B. Fundamental Processes of Contaminant Removal

Microbial reduction of uranium and selenium for contaminant removal have been extensively studied (Lovley et al., 1991; Lovley and Phillips, 1992a; Lovley and Phillips, 1992b; Tomei et al., 1992; Tomei et al., 1995). Contaminant removal of chromium by microbial action has also been studied to a lesser degree (Fude et al., 1994). However, reduction and removal of molybdenum or arsenic by microbial processes is not well understood. For example, microbial reduction of molybdenum (as soluble Mo^{6+}) has only been demonstrated to produce Mo^{5+} , which is also soluble. In order for molybdenum to be precipitated from solution in the microbial barrier, it must be reduced all the way to

Mo⁴⁺ so that insoluble compounds such as MoS_{2(s)} will be formed. Therefore, an important development need is to understand the fundamental processes of microbial reduction of all the contaminants of concern at UMTRA sites, especially for molybdenum and arsenic.

In addition, most studies on the microbial reduction of uranium has taken place in relatively simple systems (i.e., the U-C-O-H system) in laboratory reactors. However, the chemistry of UMTRA groundwaters is a great deal more complex (DOE/UMTRA, 1991a). Therefore, there is a need to study the microbial reduction of uranium and other contaminants in complex water systems which closely resemble the chemistry of UMTRA groundwaters.

There is also the concern that reducing conditions may increase the solubility of some contaminants (especially As). As previously described, some studies have indicated that arsenic is sorbed onto solid ferric iron particles under oxidizing conditions and that a change to reducing conditions in the system (with subsequent reduction and dissolution of the iron particles) may actually increase arsenic solubility by releasing it into solution (Masscheleyn et al., 1991). This process must be further studied to ensure that the microbial barrier will be removing all contaminants of concern from solution. Also, the presence of organic compounds in the barrier may increase (or decrease) the solubility of contaminants. For example, methylated arsenic oxyacids can be produced by a variety of organic compounds and their presence has been reported in a wide range of natural waters (Masscheleyn et al., 1991). It is clear that some of the basic processes that may occur in the barrier are not yet well understood and will require further study.

In addition, field-scale studies need to be set up to prove that this technology can work under the conditions encountered at actual UMTRA sites. Most microorganisms that will be utilized in a microbial barrier sustain their best growth at a temperature of approximately 30°C (Zehnder, 1988). However, the temperature of groundwater at UMTRA sites is typically around 15°C. This issue has not been addressed in laboratory studies. Accumulation of contaminants in the barrier over a period of many years may also be a problem. For example, molybdenum has been shown to be an inhibitor of sulfate-reducing bacteria at concentrations of approximately 20 mM. Build-up of molybdenum or other contaminants during the lengthy operation of the barrier may cause toxicity problems to the bacteria. However, this most likely will not be a major concern in the barrier because concentrations of contaminants in UMTRA groundwater are relatively low (~0.1-10 mg/l) compared to the levels that are needed to inhibit bacteria (typically in the mM range).

C. Supply of Substrate, Nutrients and Electron Acceptor

Supplying the bacteria with an organic substrate may be a difficult problem. The substrate must be long-lasting, inexpensive and capable of supplying low molecular weight organic acids to sulfate-reducing bacteria. Since pure pyruvate, lactate or acetate is relatively expensive, it may be necessary to grind and mix hay, alfalfa, straw or sawdust with the barrier matrix (coarse sand or gravel) and allow a mixed culture of bacteria to produce the required organic acids by fermentation or incomplete oxidation. Finely ground sawdust was successfully used as a substrate in an anaerobic subsurface microbial barrier which was designed to denitrify septic tank effluent in a drainfield (Robertson and Cherry, 1995). In this research, the authors showed that significant sulfate reduction also occurred in addition to nitrate reduction which would indicate that sulfate-reducing bacteria were able to grow in the barrier. However, a suitable substrate has not yet been identified for this particular application so this is an area where further development is needed. Addition of nutrients, especially phosphorous, will probably also be required since the concentration of phosphate in the groundwater at mill tailings sites is usually not sufficient to support high-rate bacterial growth (DOE/UMTRA, 1991b).

A readily available primary electron acceptor will not be a problem since the groundwater at most mill tailings sites have sulfate concentrations between 1000-13,000 mg/l (DOE/UMTRA, 1991b). However, the pH of the groundwater at mill tailings sites is a concern since it is typically between 3 and 4 immediately below the mill tailings piles, which is considered too low for optimal growth of most microorganisms (especially sulfate-reducing bacteria). Fortunately, at most sites, the pH is neutralized as the groundwater moves down-gradient. Therefore, care will have to be taken to construct the barrier a sufficient distance down-gradient from the mill tailings piles so that the pH will be closer to the optimal range for the bacteria (a pH between 5.5 and 8.5 for sulfate-reducing bacteria). It is also thought that many microorganisms will live in microniches in the soil where the pH will be higher than the overall pH of the groundwater so microorganisms may be able to grow in groundwater with a lower than optimal pH (Zehnder, 1988).

D. Physical Emplacement and Configuration of Barrier

At UMTRA sites, a microbial barrier would need to be placed in a vertical configuration as shown in Figure 1 (Section 1). In this configuration, the microbial barrier would be placed in an excavation constructed with the use of sheet piles or other trenching methods in the groundwater system down-gradient from a disposal cell in the path of the contaminant plume. With existing trenching techniques, the depth of the barrier would be

limited to 30'-40'. Therefore, the barrier would be limited to situations where the contaminants are migrating at a shallow depth primarily in the horizontal direction. Further development is required to determine methods which may be used to emplace the barrier material at depths greater than 30'-40' so that the barrier system may be applicable at a larger number of sites. For example, deep soil mixing has been used for remediation of contaminated soil for many years. Deep soil mixing is a technology used to stabilize a contaminant within a soil matrix. An auger is drilled into the soil and then is extracted to approximately one-half the depth of the hole. After the auger is partially extracted, it is reinserted while initiating injection of stabilization material. Reinsertion continues until the auger reaches the bottom of the hole. The process is repeated in a predetermined pattern until the entire volume of soil has been stabilized. Conceivably, this process (or other existing technologies) could be modified to inject reactive barrier material (or organic substrate and nutrients) into the soil system to create a permeable barrier for groundwater remediation.

Work is also needed to develop technologies for emplacement of the reactive barrier material in a well-mixed manner at the proper permeability. If gaps are left in the barrier material, contaminants may flow through the barrier and migrate into the aquifer system. The permeability of the barrier material is also important and must be equal to or greater than the surrounding aquifer material. If the permeability of the barrier material is too low, the groundwater may be deflected around the barrier and contaminants may migrate into the aquifer system. To maintain proper permeability in the barrier, the optimal material for the barrier will most likely be a coarse sand mixed with a finely-ground solid organic carbon source. However, studies must be conducted to determine the proper mixture of the barrier material and the best methods for emplacement of this material.

E. Effective Capture Zone

To effectively remediate a contaminant plume, the vertical microbial barrier must be properly designed so that the entire plume will pass through the barrier. If the plume is very wide and/or very deep, a large barrier may be impractical. To get around this problem, the barrier system may be designed with low conductivity cutoff walls to focus the groundwater flow through the barrier which would allow a smaller reactive barrier to actually treat the plume. This approach is known as the 'funnel-and-gate' system (Starr and Cherry, 1994). Several different configurations to the 'funnel-and-gate' approach are shown in Figure 8 at the end of this section. Groundwater and contaminant flow through various 'funnel and gate' system configurations has been simulated with 2D and 3D groundwater flow models to determine if a selected vertical barrier design will effectively

capture the entire contaminant plume. The results of one simulation (using FLONET, a 2D model) is shown in Figure 9.

In designing 'funnel-and-gate' systems, two conflicting factors must be considered in evaluating different configurations. On one hand, the flow through the gate should be maximized to make the capture zone as large as possible. On the other hand, the residence time in the barrier should be long enough to achieve effective removal of the contaminants. This must be balanced with attempts to minimize the cost of the microbial barrier by using as little material as possible.

Studies concerning the flow characteristics in and around barriers is a somewhat neglected area of research and further development is needed. In order to implement microbial barriers (or any kind of subsurface reactive barriers) at UMTRA sites, detailed hydrologic characteristics of the aquifers at those sites is required. A comprehensive program must be undertaken to determine which UMTRA sites are candidates for barrier technology based on the hydrologic characteristics of the aquifers (and the nature of contamination). At the sites that are selected for implementation of a microbial barrier, a detailed design of the barrier system making use of 2D or 3D groundwater models must be undertaken to determine if the contaminant plume can be captured and mitigated.

F. Transient Changes in Groundwater Chemistry

Changes in the groundwater chemistry is an important issue when considering the use of a microbial barrier. Although there are many potential changes that could be considered, this discussion will focus on two important variables, Eh and pH. The Eh and pH of the system are important for two reasons. First, the solubility of heavy metal contaminants is generally determined by the Eh and pH of the system. Second, the viability of the microorganisms (and especially the microbial enzymes) are also affected by changes in the Eh and pH of the groundwater. Under normal conditions, the groundwater in the barrier will be maintained under reducing conditions (low Eh) near a neutral pH (pH ~6-8).

The Eh of a system can greatly affect the solubility of heavy metals. For example, uranium and molybdenum, like many heavy metals, can attain multiple oxidation states. In general, these heavy metals are soluble in their higher oxidation state, and insoluble in their reduced state. Uranium, for example, is soluble as U(VI) and much less soluble as U(IV) while molybdenum is soluble as Mo(VI) or Mo(V) and less soluble as Mo(IV) (Brookins, 1987). If a pulse of oxygenated water (high Eh) enters the barrier, the reducing conditions which normally will be maintained in the barrier will be disrupted and many heavy metals

such as U and Mo may be dissolved back into the groundwater. This is also true for Se, As, and Cr.

The pH of the system will also affect the solubility of heavy metals. Many heavy metals are very soluble at low pH but form insoluble hydroxide precipitates at high pH. If a pulse of low pH (acidic) water enters the barrier, heavy metal precipitates that have been deposited in the barrier may be dissolved back into the groundwater causing recontamination of the aquifer.

A properly operating microbial barrier will maintain a population of anaerobic bacteria in a low oxygen environment represented by a low Eh. Most anaerobic bacteria will not survive or will become dormant in the presence of oxygen. Therefore, a pulse of oxygenated water (high Eh) may disrupt the microbial population in the barrier causing inactivation or death of the microorganisms.

The pH of the system will also be important to the viability of the microorganisms in the barrier. Most of the important bacteria in the system are neutrophilic which means they prefer a neutral pH (usually between 5.5 to 8.5). At low pH or high pH values, the enzymes that are responsible for catalyzing metal reduction reactions will be denatured and will no longer function (Lovley and Phillips, 1992; Zehnder, 1988). If this occurs, contaminants will no longer be removed from the groundwater in the barrier.

Transient changes in groundwater chemistry will affect the operation of the microbial barrier. However, it should be noted that changes in groundwater chemistry will also affect other groundwater remediation systems including other types of permeable, reactive, subsurface barriers and pump and treat systems. The impact of transient changes in groundwater chemistry on the operation of the microbial barrier must be studied in greater detail before a microbial barrier can be implemented at an UMTRA site.

G. Replacement of Barrier Material

In the ideal situation, a permeable reactive subsurface barrier would be installed and operated for the entire lifetime of a remediation project (100 years for an UMTRA site) with very little maintenance. However, in an actual installation, it might be necessary to replace the reactive material in the barrier one or more times during the duration of the project. In the case of a microbial barrier, this need may arise for two reasons. First, it may be necessary to replace the substrate (i.e., carbon source) or nutrients (i.e., nitrogen and phosphorous) in the barrier if these materials have been consumed by the microbes before the end of the project. Second, it may be necessary to excavate the barrier material if it is believed that the precipitated heavy metal contaminants might recontaminate the

aquifer by dissolving back into the groundwater (especially as groundwater chemistry changes occurred in the aquifer).

Consumption of the substrate and nutrients within the microbial barrier points out the need to obtain kinetic data concerning the rate of use of these materials by microorganisms. As discussed earlier, the most important microorganisms in the microbial barrier will probably be sulfate-reducing bacteria. A significant amount of data has been collected on the kinetics of sulfate-reducing bacteria (Magee et al., 1978; Middleton and Lawrence, 1977; Thomson, 1987; Tucker, 1994). By making use of this kinetic data along with hydrologic and contaminant data from the aquifer, the lifetime of a barrier could be predicted and the substrate replaced at the appropriate time. Examples of kinetic data which have been collected in previous research is shown in Table 1. By using these kinetic coefficients along with the appropriate rate expressions (i.e., the Monod equation), it could be determined when substrate needed to be replenished.

The barrier material may also need to be excavated and replaced if it is determined that the precipitated contaminants are not stable as conditions change in the barrier. This issue has been discussed above. At this time, this stability issue is not well understood and requires further study. This stability problem can be illustrated by examining the uranium reduction process. As previously described, several studies have shown that sulfate-reducing bacteria can reduce U(VI) to U(IV) which is precipitated as uraninite ($\text{UO}_{2(s)}$) (Lovley et al., 1991; Lovley and Phillips, 1992a; Lovley and Phillips, 1992b; Tucker, 1994). However, $\text{UO}_{2(s)}$ exists in both crystalline or amorphous forms. As shown in Figure 10, the stability field for the crystalline form of $\text{UO}_{2(s)}$ is much larger than the stability field for the amorphous form. This means that crystalline $\text{UO}_{2(s)}$ is more stable than amorphous $\text{UO}_{2(s)}$ under various Eh and pH conditions. However, it is difficult to predict whether crystalline or amorphous $\text{UO}_{2(s)}$ will be formed by microbial reduction and to determine the actual stability of the precipitate. If further research shows that the precipitated contaminants are relatively unstable, the barrier material (along with the precipitated metal contaminants) will have to be periodically excavated and disposed of in an approved manner, possibly with the recovery of the precipitated metals. Alternatively, if the precipitated species of the heavy metal contaminants were shown to be stable, the barrier material could be left in place throughout the lifetime of the project.

H. Monitoring of the Microbial Barrier

Monitoring of the microbial barrier system will be an important issue when this type of system is installed in the field since proper monitoring is the only way to determine if the barrier is effectively removing the contaminants of concern from groundwater. There

are three areas that could potentially be monitored: 1) groundwater up-gradient from the barrier, 2) soil conditions and microbial activity inside the barrier, and 3) groundwater down-gradient from the barrier. A reliable, inexpensive monitoring system must be developed to monitor the microbial barrier to ensure proper operation (i.e., contaminant removal).

A series of monitoring wells could be installed up-gradient from the barrier. The purpose of these wells would be to monitor the conditions in the groundwater entering the barrier. Two critical parameters that should be monitored include the Eh and pH of the groundwater since changes in these parameters can upset the operation of the barrier for the reasons that have been discussed previously. First, the solubility of heavy metal contaminants is generally determined by the Eh and pH of the system. Second, the viability of the microorganisms (and especially the microbial enzymes) are also affected by changes in the Eh and pH of the groundwater. The up-gradient monitoring wells could be used to determine if these two parameters are in the desired operating range for the barrier (i.e., low Eh and near neutral pH).

Soil conditions and microbial activity within the barrier could also be monitored. Parameters to be monitored could include microbial population density, microbial classification, soil permeability, soil organic content, substrate concentration, total nitrogen, pH, Eh, and the concentration of the heavy metal contaminants of concern. Since analysis costs for these parameters will be relatively expensive, soil should be collected from the barrier and analyzed only three or four times a year. If it is established after a period of time that the barrier is operating properly, analysis of the conditions within the barrier can be performed on a less frequent basis.

Monitoring wells should also be installed down-gradient from the barrier to monitor groundwater quality. The samples should be analyzed for the contaminants of concern at each particular site. Other parameters, such as the sulfate concentration, could also be monitored to determine if a specific population of bacteria (i.e., sulfate-reducing bacteria) that are important to the operation of the barrier are functioning. Samples taken from these down-gradient monitoring wells will provide the only real verification that the barrier is operating properly.

At this time, a strategy for monitoring the performance of a microbial barrier has not been established. Variables such as the location and number of wells, frequency of sampling, type of analysis, and parameters to be measured need to be defined before a microbial barrier can be implemented at an UMTRA site.

Figure 8: Configurations of the 'Funnel-and-Gate' Approach (Starr and Cherry, 1994)

Figure 9: 'Funnel-and-Gate' System and Capture Zone (Starr and Cherry, 1994)

Figure 10: Eh-pH Diagram for the U-O₂-CO₂-H₂O System (Langmuir, 1978)

Study	k (days ⁻¹)	K_s (mg COD/L)	Y (mg cells/mg COD)	k_d (days ⁻¹)
Tucker (1994)	4.7	140.0	0.170	0.072
Middleton and Lawrence (1977)	7.1	99.0	0.060	0.0
Thomson (1987)	16.9	2.8	0.065	-

k : maximum rate of substrate utilization

K_s : half-velocity constant

Y : specific yield

k_d : endogenous decay coefficient

Table 1: Kinetic Coefficients for the Growth of Sulfate-reducing Bacteria from Three Studies (Tucker, 1994)

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Section 5

Conclusions

The microbial barrier is a promising alternative for groundwater remediation at certain sites. Currently, the state of this technology can be summarized as follows:

1. Dissimilatory metal reduction of U(VI) to U(IV) with the subsequent precipitation of uranium from solution as uraninite ($\text{UO}_2(\text{s})$) has been demonstrated at the laboratory-scale in relatively simple water systems (i.e., U-C-O-H). Several microorganisms have been identified that have the capability to microbially reduce uranium including the sulfate-reducing bacterium, *Desulfovibrio desulfuricans* and the iron-reducing bacteria, *Geobacter metallireducens* and *Shewanella putrefaciens*.
2. Dissimilatory metal reduction of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) with the subsequent precipitation of selenium from solution as elemental selenium (Se^0) has been demonstrated in the laboratory by several microorganisms including *Desulfovibrio desulfuricans* and *Wolinella succinogenes*.
3. Reduction of Cr(VI) to Cr(III) and subsequent precipitation of chromium as $\text{Cr}_2\text{O}_3(\text{s})$ as a result of strongly reducing conditions generated by microorganisms has been demonstrated in the laboratory by a consortium of sulfate-reducing bacteria and by *Escherichia coli*.
4. Generation of biogenic sulfide by sulfate-reducing bacteria with the subsequent precipitation of Cd and Mo as their sparingly soluble sulfides (i.e., $\text{CdS}(\text{s})$ and $\text{MoS}_2(\text{s})$) has been demonstrated in the laboratory. However, reduction of Mo(VI) to Mo(IV) by biogenic sulfide has not been demonstrated.
5. Reduction and precipitation of As and V by microbial processes has not been demonstrated.

However, several key issues remain to be resolved before this type of system can be implemented at a contaminated site. Some of these issues include:

1. Understanding of the fundamental processes of contaminant removal in the barrier.

2. Development of methods to supply organic substrate, nutrients, and electron acceptors for sustained growth of microorganisms in the barrier.
3. Development of methods for the physical emplacement of the barrier and configuration of the reactive bed.
4. Determination of the effective capture zone by hydrogeological modeling.
5. Determination of the effects of transient changes in groundwater chemistry.
6. Understanding of the need for or the timing of the replacement of the reactive bed.
7. Development of a monitoring program to assess the performance of the barrier.

These issues have been addressed in this report. Although some of these issues are being resolved by development activities that are currently in progress, further development will be required in order to resolve all of these questions. At this time, it is thought that the two most critical issues on which development activities should be focused on are: 1) understanding of the fundamental processes for contaminant removal in the barrier, and 2) determination of the long-term stability of the precipitated heavy metal contaminants. Development needs for implementation of the microbial barrier are summarized in Table 2.

Development Need	Description
Fundamental Processes of Microbial Reduction	Study of the fundamental processes of reduction of contaminants by microbial action (especially for contaminants other than uranium). Study of microbial reduction of contaminants in complex water systems.
Supply of Organic Substrate, Nutrients, and Electron Acceptor	Development of methods to supply the required substrates, nutrients, and electron acceptors to the microorganisms important for operation of the barrier (i.e. sulfate-reducing bacteria).
Physical Emplacement of Barrier	Development of trenching methods to place the barrier material at the proper location and with the required hydraulic conductivity.
Effective Capture Zone Analysis	Detailed modeling to design barriers with an effective capture zone while minimizing barrier material, operation, and maintenance costs.
Effects of Transient Changes in Groundwater Chemistry	Determine the effects of transient changes in groundwater chemistry (especially Eh and pH) on the remobilization of precipitated contaminants in the barrier.
Replacement of Reactive Barrier Material	Determination of the need and timing of the replacement of organic substrate in the barrier. Determination of the need and timing of the excavation of the precipitated contaminants in the barrier.
Barrier Monitoring Program	Development of a comprehensive monitoring system to assess the performance of the barrier in a cost-effective, reliable manner.

Table 2: Summary of Development Needs for Implementation of the Microbial Barrier at UMTRA Sites

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Section 6

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