Bioremediation of Arsenic, Chromium, Lead, and Mercury

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This report provides a basic orientation and current status of bioremediation for contaminants located in the subsurface. This report contains information gathered from a range of currently available sources, including project documents, reports, periodicals, Internet searches, and personal communication with involved parties. References for each case study are provided immediately following the case study. All sources are organized in alphabetical order at the end of the document. No attempts were made to independently confirm the resources used. It has been reproduced to help provide federal agencies, states, consulting engineering firms, private industries, and technology developers with information on the current status of this project.

This paper addresses the status of the application of biological treatment to clean up hazardous metals from the earth's subsurface (i.e., in situ bioremediation). The target audience includes federal and state regulators, planners, and site managers. The report is available on the Internet at www.clu-in.org/studentpapers/.

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Each participating headquarters or regional office develops and sponsors projects for student research. The projects are narrow in scope to allow the student to complete the research by working full-time during the summer or part-time during the school year. Research fellowships are available in environmental policy, regulations, and law; environmental management and administration; environmental science; public relations and communications; and computer programming and development.

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INTRODUCTION

Bioremediation technology uses microorganisms to reduce, eliminate, contain, or transform to benign products contaminants present in soils, sediments, water, and air. Evidence of kitchen middens (ancient household garbage dumps) and compost piles dates back to 6000 B.C. (NABIR Primer, 2003), demonstrating that some form of bioremediation was practiced by humans since the beginning of recorded history. Bioremediation was used over 100 years ago with the opening of the first biological sewage treatment plant in Sussex, UK, in 1891 (NABIR Primer, 2003). However, the word "bioremediation" is fairly new; first appearing in a peer-reviewed scientific literature in 1987 (NABIR Primer, 2003).

Interest in the application of bioremediation for environmental protection efforts in the United States has increased within the last decade. While it has been known that bioremediation can be a cost efficient and reliable method for removing hazardous waste from sites heavily contaminated sites with organic compounds, in the last decade researchers have also discovered that bioremediation technology can be used at sites contaminated with solvents, explosives, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) (NABIR Primer, 2003). Even more recently, researchers have discovered that microbial processes are beginning to be used to cleanup radioactive and metallic contaminants—two of the most common and most recalcitrant components of hazardous waste sites (NABIR Primer, 2003).

Metal contaminants are commonly found in soils, sediments, and water. Metal pollutants can be produced through industrial processes such as mining, refining, and electroplating. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, and complexation, and changes in valence state. These transformations affect the mobility and bioavailability of metals. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species. Fortunately, microorganisms can affect the reactivity and mobility of metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metal contamination.

Staphylococcus, *Bacillus*, *Pseudomonas*, *Citrobacteia*, *Klebsilla*, and *Rhodococcus* are organisms that are commonly used in bioremediation mechanisms (Connor, Landin, Mellor, O'Donovan, a. 1994 b. 1996). These mechanisms include bioaugmentation, in which microbes and nutrients are added to the contaminated site, and biostimulation, in which nutrients and enzymes are added to supplement the intrinsic microbes of the site (Connor, Landin, Mellor, O'Donovan, a. 1994 b. 1996). These organisms are often used in the bioremediation of cadmium (Connor, Landin, Mellor, O'Donovan, a. 1994 b. 1996). *Alcaligenes* and *Pseudomonas* have been used in the bioremediation of chromium (Connor, Landin, Mellor, O'Donovan, a. 1994 b. 1996). Likewise, organisms like *Escherichia* and *Pseudomonas* have been used in the bioremediation of copper (Connor, Landin, Mellor, O'Donovan, a. 1994 b. 1996). Currently, more research is being performed on the use of microbes to degrade metals.

It is anticipated that federal, state, and local governments and private industries will annually invest billions of dollars over the next several decades to clean up sites contaminated with hazardous waste (NABIR Primer, 2003). This investment validates the need to research the utilization of microbial processes to clean up contaminated sites.

Superfund is a federal program managed by the EPA that enforces the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) created for site identification and cleanup (USEPA Office of Solid Waste and Emergency Response, 2004). Volatile organic compounds (VOCs) and metals are the major contaminants found at Superfund sites (USEPA Office of Solid Waste and Emergency Response, 2004). As of May 2003, there were 456 federal and non-federal Superfund sites listed on the National Priorities List (NPL) that still required at least one more additional remedial action (USEPA Office of Solid Waste and Emergency Response, 2004). EPA reports that 77% of these sites need to be remediated for metals, 78% for volatile organic compounds, and 71% for semivolatile organic compounds (SVOCs). A 2001 study from the EPA predicts that within the next 10 years, an additional 23 to 49 sites will need to undergo extreme cleanup (USEPA, 2004).

Metal contaminants are not just at Superfund sites. Sites managed by the Department of Defense (DoD) and the Department of Energy (DOE) are also contaminated with metals. The DoD is responsible for cleaning up contamination at DoD facilities where numerous industrial, commercial, training, and weapons testing activities took place (USEPA, 2004). The DoD reports that approximately 9,000 of its sites require remediation (USEPA, 2004). The most prevalent groundwater contaminant groups are volatile organic contaminants and metals, which appear at 74 percent and 63 percent of DoD sites (USEPA, 2004). This further supports the need for research on the bioremediation of metals. Metals were found between 63-79 percent of the DOE sites (USEPA, 2004).

The DOE's sites contain radiation hazards, volumes of contaminated soil, and nuclear waste (USEPA, 2004). These sites account for one third of the total U.S. cleanup market (USEPA, 2004). Metals, such as lead, chromium VI, mercury, zinc, beryllium, arsenic, cadmium, and copper are abundant throughout these sites (USEPA, 2004). Thus, it goes without saying that the bioremediation of metal contaminants can have a wide application to site cleanup.

These metal contaminants pose adverse health effects to those who live near these polluted sites. Metal waste is commonly found in soil, sediments, and water. Breathing, eating, drinking, and skin contact are all possible exposure routes for metal contaminants. Metals such as mercury, lead, and arsenic, potentially can be toxic to the kidneys, decrease mental capabilities, and cause weakness, headaches, abdominal cramps, diarrhea, and anemia (USEPA, 2004). Chronic exposure to these pollutants can cause permanent kidney and brain damage (USEPA, 2004).

In situ bioremediation is a technique that can be used to reduce the spread of metal contaminants by applying biological treatment to hazardous chemicals in soil and groundwater (Connor, Landin, Mellor, O'Donovan., a. 1994 b.1996). In situ bioremediation has the ability to transform contaminants to less toxic compounds, making this a promising environmental cleanup technique (National Research Council, 1993). It accelerates contaminant desorption and dissolution by treating pollutants close to their source. Methods such as pump-and-treat only remove or destroy contaminants in groundwater, but not those contaminants sorbed in soil or solids in the aquifer (National Research Council, 1993).

Although the use of bioremediation is rapidly growing, in situ bioremediation of metals is not widely understood (National Research Council, 1993). The many intricacies of microbial metabolism related to metals have not been grasped fully because successful usage of microbes to clean up metals is a complex matter. Furthermore, the process of verifying whether or not

complete bioremediation has actually occurred (meaning the metal contaminant changed forms via abiotic chemical reactions) is an issue researchers are still struggling with in the labs. Demonstrating that lab-grown microbes have the potential to degrade the contaminant is not enough to sufficiently prove that microorganisms can completely clean up a site (National Research Council, 1993). Moreover, there is still a need for researchers to verify that the bioremediation of metal contaminants is a permanent process.

Chemical Process of Bioremediation

Bioremediation involves the use of microorganisms to aid with the destruction of contaminants, a process called microbial metabolism. This process involves biochemical reactions or pathways in an organism that result in activity, growth, and reproduction of that organism. Chemical processes involved in microbial metabolism consist of reactants, contaminants, oxygen, or other electron acceptors, which convert metabolites to well-defined products. The microbial metabolism system enables organisms to retrieve carbon, electrons, and other vital components to survive. In some cases, the contaminant may be transformed while the microorganisms seek other sources of energy or carbon. This reaction is described as "cometabolism," during which the transformation of the contaminant yields little or no benefit to the cell. Secondary utilization is another way to describe a non-beneficial biotransformation. This transformation of the contaminant is an incidental reaction catalyzed by enzymes present in the cell's metabolic system (National Research Council, 1993).

Aerobic respiration is a process that involves microorganisms using oxygen to oxidize a carbon source that may exist in a contaminant. Many microorganisms use aerobic respiration as a way to destroy organic contaminants. Under aerobic conditions, a drop in oxygen concentration occurs when microbes are active, which is instrumental in the reproduction of some living organisms. During this process, oxygen is reduced resulting in the formation of water.

Microorganisms that can live without oxygen use anaerobic respiration for a metabolic process. Unlike aerobic respiration, where oxygen serves as a main electron acceptor, anaerobic respiration uses inorganic compounds—such as nitrate, sulfate, and iron—as electron acceptors. Inorganic molecules, such as ammonium, nitrite, and reduced iron, can serve as electron donors. Once these molecules are accepted as electron donors, they are oxidized and their electrons are transferred to electron acceptors (usually to oxygen) to produce energy for cell synthesis. Microorganisms whose primary electron donor is an inorganic molecule must receive their carbon source from carbon dioxide—a process called carbon dioxide fixation. Nitrogen gas, hydrogen sulfide, and reduced forms of metals are other byproducts of anaerobic respiration. In circumstances where metals are used as electron acceptors by anaerobic organisms, the metal precipitates, which decreases its concentration and mobility in groundwater (National Research Council, 1993). In general under anaerobic conditions, concentrations of electron acceptors (such as nitrate and sulfate) will decrease. Today research is focusing on metal contaminants that convert into a precipitate form when exposed to microorganisms. Metals that undergo this procedure offer promising solutions concerning the removal of persistent metal contaminants in the environment.

Fermentation is another type of metabolism mechanism that assists with the conversion of a contaminant into a less harmful compound. Fermentation is a metabolic method that occurs without oxygen through a series of internal electron transfers catalyzed by the microorganisms. Fermentation products, like acetate, propionate, ethanol, and hydrogen, are examples of elements

that can be biodegraded by bacteria (National Research Council, 1993). Some bacteria species can convert these products to carbon dioxide, methane, and water. This conversion can assist with the cleanup of some contaminant sites.

Another method of microbial metabolism is reductive dehalogenation. Reductive dehalogenation is potentially important in the detoxification of halogenated organic products (National Research Council, 1993). This method involves microbes catalyzing a halogen atom on the contaminant and replacing it with a hydrogen atom. This reaction adds two electrons to the contaminant molecule allowing the molecule to convert into a reduced form (National Research Council, 1993). In order for this method to occur, an electron donor must be taken from a different source other than a halogenated contaminant. Lactate, acetate, methanol, and other low molecular weight organic compounds may serve as possible electron donors. Reductive dehalogenation is not commonly known to generate energy for microorganisms, but it provides a way to eliminate toxic material from the microorganism's cell (National Research Council, 1993).

Mobility of Metal Contaminants

As stated above, microbes can convert contaminants to less harmful products. However, in addition to their conversion ability, they can cause contaminants to be demobilized (National Research Council, 1993). The immobility of metals is primarily caused by reactions that cause metals to precipitate or chemical reactions that keep metals in a solid phase (Evanko and Dzombak, 1997). Chemical and physical properties affect the mobility of metals in soils and groundwater. Under acidic conditions (pH ranges between 4.0-8.5), metal cations are mobile while anions tend to transform to oxide minerals. At high pH levels, cations adsorb into mineral surfaces and metal anions are mobilized. Hydrous metal oxides of iron, aluminum, and manganese can affect metal concentrations because these minerals can subtract cations and anions.

"Biocurtain" is a term used to describe the process by which large amounts of biomass stop or slow contaminant movement. The biomass can then absorb hydrophobic organic molecules (National Research Council, 1993). A large biomass also can hinder the migration of a contaminant.

When a microorganism oxidizes or reduces species, this reaction causes metals to precipitate (National research Council, 1993). Mercury is an example of a metal that can be precipitated. The process begins when mercury (Hg^{2+}) is reduced to mercuric sulfide causing mercury to transform to a precipitated form (National research Council, 1993). Chromium is another metal that can convert to a precipitated form with the use of microorganisms. The process involves the reduction of hexavalent chromium (Cr^{6+}) to trivalent chromium (Cr^{3+}), which then can precipitate to chromium oxides, sulfides, or phosphates (National Research Council, 1993). Research today is focusing on other metal and radioactive contaminants that can undergo precipitation processes.

Superfund, DoD, and DOE Sites

Today, federal, state, and local governments spend billions of dollars on research efforts to discover effective and economical ways to remove contaminants from the environment. According to the EPA, metals (excluding radioactive metals) are present at three-quarters of the nation's Superfund sites (EPA, 2004). EPA estimates that 77,000 known sites still require remediation and new discoveries of contaminated properties are expected to continue (USEPA,

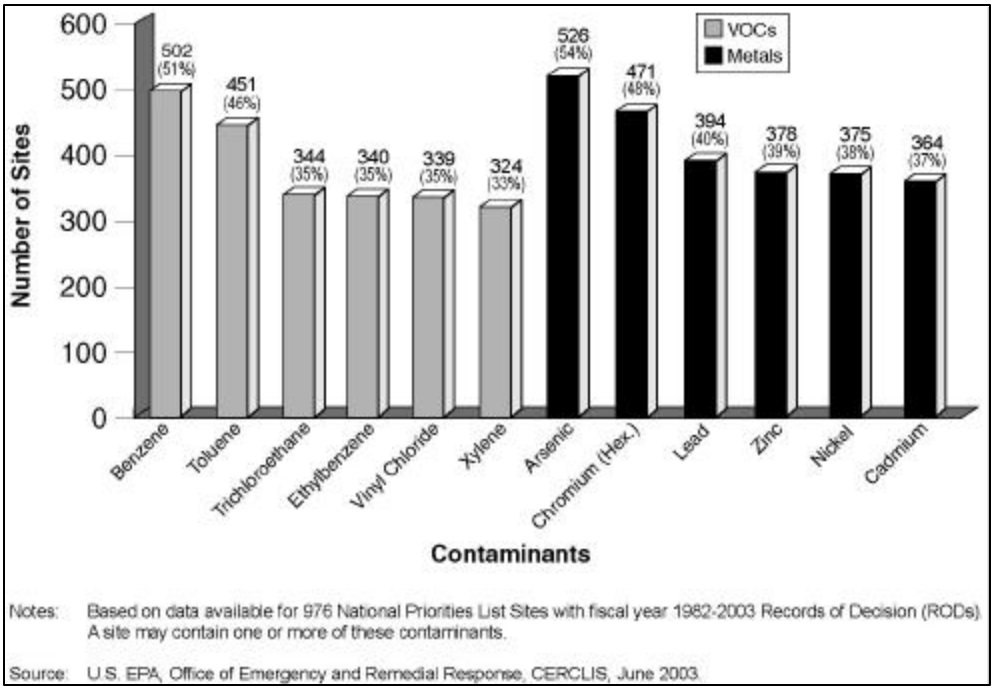
2004). It has been estimated that an additional 217,000 additional contaminated sites are likely to be discovered during the next 30 years. Because of these numbers, extensive research on the chemical processes of bioremediation and on how metals can be effectively remediated is being explored.

Superfund Sites

The Superfund Program, administered by EPA, is the federal program to clean up releases at abandoned or uncontrolled hazardous waste sites. It was established by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). CERCLA was altered by the uperfund Amendments and Reauthorization Act of 1986 (SARA) (USEPA, 2004). Among other changes, SARA supports the application of new technology that achieves permanent results and research to "improve site investigation and cleanup methods."

As of September 2003, EPA had listed 1,244 Superfund sites on the NPL (USEPA, 2004). EPA reports that 78% of these sites need to be remediated for volatile organics, while 77% need to be remediated for metals, and 71% for semivolatiles or organics (USEPA, 2004). Of the 12 contaminants most frequently found at Superfund sites, half are metals (primarily arsenic, chromium, lead, zinc, nickel, and cadmium). Arsenic, chromium, and lead are the most prevalent metals found at Superfund sites (USEPA, 2004). EPA estimates that at 456 NPL sites with planned remedial actions, about 66 million cubic yards of soil, sediment, and sludge are contaminated with metals (USEPA, 2004). These data support the extreme need to discover permanent solutions to remove hazardous materials and metal contaminants from the environment.

Figure 1 shows the frequencies of the most common contaminants at NPL sites (USEPA, 2004).

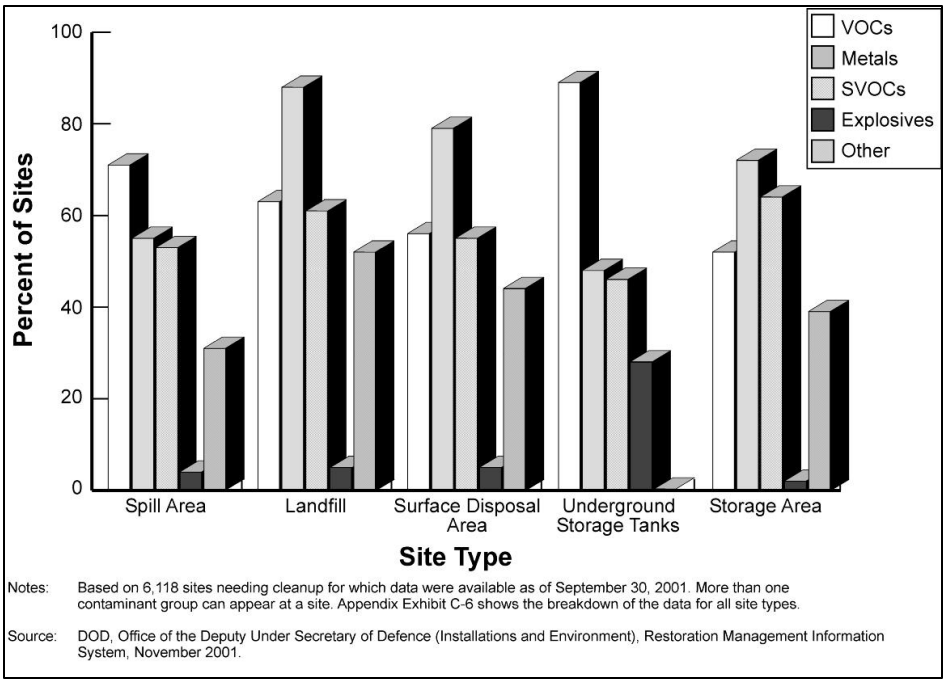


Department of Defense

The Department of Defense (DoD) is comprised of the Air Force, Army, and Navy. This Department holds the responsibilities for protecting the borders of the United States and also for cleaning up waste remaining from industrial, commercial, training, and weapons activities. The DoD estimates that as of September 2003, almost 6,400 contaminated sites were in various stages of site investigation, and almost 2,700 sites were planning for or in various stages of ccleanup, bring the total number of sites yet to be cleaned up to more than 9,000. Forty-four percent of these sites are in six states: Virginia, Texas, Florida, Maryland, Alaska, and California (USEPA, 2004).

Volatile organic compounds, semivolatile organic compounds, and metals are the most prevalent types of contaminants at DoD sites (USEPA, 2004). Volatile organic compounds are present at 64% of DoD sites, semivolatile contaminants are present at 57% of these sites, and metals are present at 72% of these sites (USEPA, 2004). In FY 2004, DoD allocated 20% of its cleanup budget to evaluate and clean up properties that are to be transferred to other federal, state, or local government agencies or private parties.

Figure 2 displays the frequency of major contaminant groups (VOCs, metals, SVOCs, explosives, and other) for the most common DoD site types needing cleanup (USEPA, 2004).



Notes: Based on 6,118 sites needing cleanup for which data were available as of September 30, 2001. More than one contaminant group can appear at a site. Appendix Exhibit C-6 shows the breakdown of the data for all site types.

Source: DOD, Office of the Deputy Under Secretary of Defence (Installations and Environment), Restoration Management Information System, November 2001.

Department of Energy

The Department of Energy has more than 10,000 contaminated sites for which it is responsible for cleanup. More than 5,000 of these have been remediated, leaving about 5,000 sites on 39 DOE installations and other properties. DOE also is responsible for 19 sites currently on the NPL. The Rocky Flats Environmental Technology site in Colorado, the Idaho National Engineering Laboratory in Idaho, the Savannah River Site in South Carolina, the Oakridge Reservation in Tennessee, and the Hanford Reservation in Washington are examples of DOE installations that contain sites that require remediation (USEPA, 2004).

Radionuclides, metals, and dense nonaqueous phase liquids are the major contaminants. Prevalent metal contaminants include lead, chromium VI, mercury, zinc, beryllium, arsenic, cadmium, and copper (USEPA, 2004). One main challenge faced by DOE is the lack of technology available for some of the main contaminants present on the sites, especially radionuclides. DOE plans to complete active remediation of most of its sites by 2035 (USEPA, 2004). However the monitoring and groundwater treatment programs may continue beyond this proposed cleanup date (USEPA, 2004)

Figure 3 shows the percentage of contaminated groups that need to be remediated in NPL, RCRA, DOD, and DOE sites (USEPA, 2004). Metals are present a many hazardous waste sites. For example, metals (not including radioactive metals) are present at 77% of NPL sites.

Figure 3. Contaminant Groups to be Remediated

Remediation Program	Percent of Sites		
	VOCs	Metals	SVOCs
NPL Sites	78%	77%	71%
RCRA Corrective Action Sites	67%	46%	32%
DOD Sites	64%	72%	57%
DOE Sites	38%	55%	38%

Notes:
1)DOE figures for VOCs and SVOCs are combined. 90% of DOE sites contain radioactive elements.
2)About 19% of DOD sites yet to be investigated and/or cleaned up may contain unexploded ordnance or waste military munitions.

MECHANISMS FOR METAL REMEDIATION

Today's industrial world has contaminated our soil, sediment, and water sources with hazardous materials. Metal waste is often a result of industrial activities, such as mining, refining, and electroplating. Mercury, arsenic, lead, and chromium are often prevalent at highly contaminated sites. This fact holds significant challenges for industries because these metals are difficult to remove. Therefore researchers and industries are researching metals that undergo methylation, complexation, or changes in valence state. These are noteworthy processes because they aid with the mobility and bioavailability of metals (NABIR, 2003). There is a large interest in microorganisms that can facilitate with the transformation and the removal of the metal contaminant.

Remediation of metals often involves five general approaches: isolation, immobilization, mobilization, physical separation, and extraction (Evanko and Dzombak, 1997). Immobilization and mobilization involve bioremediation processes. Industries use a combination of more than one approach to properly treat metal-contaminated sites. The combination of the approaches can be cost-effective.

Immobilization

Immobilization is a technique used to reduce the mobility of contaminants by altering the physical or chemical characteristics of the contaminant. This remediation approach can utilize microorganisms to immobilize metal contaminants. Most immobilization technologies are performed in situ or ex situ. In situ means the treatment is below the ground. Ex situ means the waste is excavated or pumped above the ground for treatment (USEPA, 2001). Ex situ processes are effective for contaminants located in large volumes of waste.

The immobilization technique is based on the characteristics of the site. In situ stabilization and solidification is most beneficial for sites with contamination located less than 8-10 feet below the ground. In situ is preferred because it requires less labor and energy than ex situ. Immobilization is usually accomplished by physically restricting contact between the contaminant or by chemically altering the contaminant (Evanko and Dzombak, 1997). Chemical reagents and bacterial reagents assist with the immobilization of metal contaminants.

Most sites contaminated with metals use the solidification and stabilization approach to immobilize metals. Thirty percent of all soil at Superfund sites uses solidification and stabilization as a remediation technique. Solidification treatment involves mixing or injecting chemical agents to the contaminated soil. The prominent mechanism by which metals are immobilized is by precipitation of hydroxides. The chemical composition of the site, the amount of water present, and the temperatures are all factors important to the successful use of the solidification/ stabilization mechanism. If these factors are not considered then they can interfere with the solidification/stabilization process by inhibiting bonding of the waste, decreasing the stability of the matrix, or reducing the strength of the solidified area. Solidification and stabilization is not an effective solidification mechanism for metal contaminants that exist as anions, such as (Cr VI) or for metals that have low-solubility hydroxides, such as mercury. This technique may not be applicable to waste sites containing organic and volatile organics. Air stripping or incineration are more effective ways to remove organics (Evanko and Dzombak, 1997).

Cement-based binders and stabilizers can be used when implementing the solidification and stabilization technique. Organic binders are applied to treat metals through polymer microencapsulation. Solidification/stabilization technologies using cement are used in industries to remediate sites containing metals like chromium, lead, arsenic, mercury, and cadmium (Evanko and Dzombak, 1997). This method also can be used for sites consisting of arsenic wastes. Bitumen, polyethylene, paraffins, waxes, and other polyolefins are materials used for the application of organic binders. Bitumen asphalt is an inexpensive and common thermoplastic binder. Polymer encapsulation involves organic materials being heated and mixed with the contaminated matrix at temperatures between 120ºC to 200ºC . The organic materials polymerize and solidify the waste and then the waste matrix is encapsulated. Polymer encapsulation requires more energy and more technical equipment than cement based solidification/stabilization operations (Evanko and Dzombak, 1997).

The stabilization and solidification technique is achieved by mixing the contaminated material with appropriate amounts of stabilizer material and water. The mixture forms a solidified matrix with the waste. The stabilization and solidification techniques can occur both in situ or ex situ. In situ is preferred for volatile or semi volatile organics. The in situ process is useful for treating surface or shallow contamination. Deep stabilization/solidification up to 50 ft can be achieved by using gauged augers (up to 3 ft in diameter each) that can treat 150-400 cubic yards per day (Evanko and Dzombak, 1997). Ex situ requires excavation, transport, and disposal of hazardous material. However it is more difficult to provide uniform and mixing through the in situ process because in situ stabilization at some sites requires cohesive soils, oily sands, and clays.

Mobilization

Microorganisms can mobilize metals through autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, methylation, and redox transformations. Heterotrophic leaching is when microorganisms can acidify their environment by proton efflux thus leading to the acidification resulting in the release of free metal cations. Autotrophic leaching is when acidophilic bacteria retrieve carbon dioxide and obtain energy from the oxidation of the ferrous iron or reduced sulfate compounds, which causes solubilization of metals. Siderophores are specific Fe (III) ligands and are able to bind to other metals, such as magnesium, manganese, chromium (III), gallium (III), and radionuclides, such as plutonium (IV). Methylation involves methyl groups that are enzymatically transferred to a metal, forming a number of different metalloids. Redox transformations can allow microorganisms to mobilize metals, metalloids, and organometallic compounds by reduction and oxidation processes. There are various metal-mobilization techniques that can occur (Gadd, 2004). The way a particular metal becomes mobilized depends on the metal's chemical and physical characteristics.

ARSENIC

Health and Toxicology

Exposure to arsenic can induce adverse health effects and can occur in a number of ways. For example, primary copper, lead smelters and chemical manufactures release arsenic into the atmosphere (Klaasen and Watkins III, 2003). Drinking water sources usually contain a few micrograms of arsenic per liter or less. Large amounts of arsenic can be found in seafood (Klaasen and Watkins III, 2003). Occupational settings also can serve as a source of arsenic exposure. In fact, many U.S. workers at factories that produce pesticides, herbicides and other agricultural products are exposed to arsenic (Klaasen and Watkins III, 2003). Smeltering industries are also sources for high levels of arsenic fumes (Klaasen and Watkins III, 2003). These industries typically account for the excessive spread of arsenic in the environment.

Arsenic is a metal that can generate multiple adverse health effects because of the many chemical forms it takes on. For example, arsenic can appear in inorganic or organic form. Arsenic trioxide, sodium arsenite, and arsenic trichloride are the most common inorganic trivalent arsenic compounds (Klaasen and Watkins III, 2003). Neurotoxiciy of both the peripheral and central nervous systems may be due to inorganic arsenic compounds. Neurotoxicity usually begins with sensory changes, muscle tenderness, followed by progressive weakness from the proximal to distal muscle groups (Klaasen and Watkins III, 2003).

Organic forms of arsenic also may be in trivalent or pentavalent forms and may even occur in methylated forms as a result of biomethylation in soil, fresh water and sea water in humans (Klaasen and Watkins III, 2003). Pentavalent inorganic forms of arsenic include arsenic pentoxide, arsenic acid, and arsenates, such as lead arsenate. Pentavalent arsenic forms affect human enzyme activity (Klaasen and Watkins III, 2003).

Acute illnesses from arsenic exposure consist of fever, anorexia, melanosis, cardiac arrhythmia, and eventually cardiovascular failure (Klaasen and Watkins III, 2003). A few days after exposure to arsenic, anemia, and granulocyopenia will appear in the body (Klaasen and Watkins III, 2003). Exposure to arsenic can inhibit succinic dehydrogenase activity and uncouples oxidative phosphorylation. Uncoupled oxidative phosphorylation is process that results in the stimulation of mitochondrial ATPase activity (Klaasen and Watkins III, 2003).

Inhibition of mitochondria function also can be caused by arsenic exposure. Mitochondria functions can be inhibited two ways: arsenic can compete with a phosphate during oxidative phosphorlation and by inhibiting the reduction of NAD (Klaasen and Watkins III, 2003). Inhibition of mitochondria respiration results in a decrease in cellular production of ATP. The ingestion of large doses (70-180 mg) of arsenic may cause fatal health problems. Chronic exposure to arsenic produces changes in skin epithelium. One to two weeks of exposure results in sensory loss in the peripheral nervous system.

Trivalent compounds of arsenic are the most toxic forms of arsenic (Klaasen and Watkins III, 2003). High doses of inorganic arsenic compounds given to a pregnant experimental animal resulted in various malformations in the fetuses and offspring. However, these effects have not been recognized in humans with excessive occupational exposures to arsenic (Klaasen and Watkins III, 2003). Studies show a possible association between inhalation exposure to arsenic

and skin and lung cancer. Some studies indicate that arsenic causes cancer of internal organs from oral digestion (Klaasen and Watkins III, 2003).

Chemical and Physical Characteristics

Arsenic appears in Group V of the periodic table (ATSDR, 1999). Although it appears in Group V it is semi metallic, in this document it will be addressed as a metal contaminant. It exists in four oxidation states: -3, 0, +3, +5 (ATSDR, 1999). The elemental form of arsenic is As (0). This compound appears as yellow and metallic gray (ATSDR, 1999). The metallic gray form is the stable form of arsenic.

In an aerobic environment, As (V) is dominant. Arsenate ($As0_4^{3-}$) and its various protonation states, H_3As0_4, $H_2As0_4^-$, $HAs0_4^{2-}$, $As0_4^{3-}$ precipitate when metal cations are present (Evanko and Dzombak, 1997). Metal arsenate complexes are stable under specific conditions. Arsenic (As (V)) possesses the ability to co-precipitate with or absorb into iron oxyhydroxides under acidic and moderately reducing conditions. Co-precipitates are immobile under acidic and moderately reducing conditions. However, arsenic mobility increases as pH increases (Evanko and Dzombak, 1997).

Many arsenic compounds absorb strongly to soils and therefore travel short distances in groundwater and surface water. Under reducing conditions, arsenic dominates when it exists as an arsenite (AsO_3^{3-}) and in its protonated forms, H_3As0_3, $H_2As0_3^-$, and $HAs0_3^{2-}$. Arsenite can absorb or co-precipitate with metal sulfides and has a high attraction to other sulfur compounds (Evanko and Dzombak, 1997). Under extreme reducing conditions, elemental arsenic and arsine AsH_3 may be present.

Arsenic is often present in its anionic form because it does not from complexes with anions such as Cl^- and $S0_4^{2-}$ (Evanko and Dzombak, 1997). Biotransformation through methlylation of arsenic can result in methylated compounds of arsine, such as dimethyl arsine $HAs(CH_3)^2$ and trimethylarsinic acid (CH_3) $As0_2H_2$ and dimethylarsenic acid $(CH_3)^2$ $As0_2H$ (Evanko and Dzombak, 1997).

Studies suggest absorption and co-precipitation with hydrous iron oxides under most environmental conditions are the most effective removal mechanisms of this complex hazardous material. Under low amounts of reactive metals in soil, arsenates can be leached easily. As (V) can be mobilized under reducing conditions (Evanko and Dzombak, 1997). Reducing conditions consisting of alkaline and saline, promote the formation of As (III) with the presence of organic compounds that form compound units with arsenic (Evanko and Dzombak, 1997).

Case Study: Mobilization of Arsenite by Dissimilatory Reduction of Absorbed Arsenate (Zobrist, 2000)

This study experiments with cell suspensions of Sulfurospirillum barnessii, a bacterium that reduces arsenate to arsenite.

Introduction

Biological processes aid with the facilitation and mobilization of arsenic. This experiment demonstrates that ferric oxide or oxyhydroxide phases are buried in sediments, and reductive dissolution of ferric oxides can release arsenic and other trace metals into the soil. (Zobrist,

2000). Solubilization of As (V) occurred when Fe (III)-respiring bacterium was incubated with contaminated lake sediments.

Research is discovering anaerobic bacteria species that can achieve respiratory reduction of As (V) to As (III) (Zobrist, 2000). Respiratory reduction of As (V) to As (III) is called dissimilatory reduction and involves two bacteria strains, *Sulfurospirillum arsenophilum* and *Sulfurospirillum barnesii* (Zobrist, 2000). This experiment studies Fe (III) respiring *Shewanella* alga, an organism that does not respire As (V), but can release As (V) and Fe (II) into solution (Zobrist, 2000).

Materials and Methods

The preparation of ferric and aluminum hydroxides involves the use of a 2-line ferrihydrite that was synthesized in a polyethylene vessel under a nitrogen (N_2) atmosphere (Zobrist, 2000). All chemicals in this experiment were made with a reagent grade. The water in this study was double-deionized and passed through a Milli-Q-System (Zobrist, 2000). Furthermore, gas from all the solutions was removed, except for analytical reagents (Zobrist, 2000).

Bacteria

S. barnessii strain was grown in a batch culture with nitrate. Nitrate acts as the electron acceptor; this situation allows the culture cells to reduce As (V) (Zobrist, 2000). Nitrate was chosen as the electron acceptor in the presence of As (V) or Fe (III) (Zobrist, 2000). The cells were harvested by centrifugation and washed twice with a solution consisting of a HEPES buffer at a pH of 7.3 and neutral salts (Zobrist, 2000).

Conclusion

The cell suspensions of *S. Barnessi* were able to reduce arsenate to arsenite. The reduction of Fe (III) in ferrihydrite to soluble Fe (II) occurred with the cultured cells (Zobrist, 2000). The rate of arsenate reduction was affected by the method that involved arsenate becoming associated with the mineral phases and the binding with the arsenate (Zobrist, 2000). The extent of the release of arsenite was regulated by the absorption of arsenite into the ferrihydrite or aluminia phases (Zobrist, 2000). These results help with understanding the mobilization of arsenic in large aquifers.

Case Study: A New Chemolithoautotrophic Arsenite-Oxidizing Bacterium Isolated from a Gold Mine: Phylogenetic, Physiological, and Preliminary Biochemical Studies (Santini, Sly, Schnagl, and Macy, 2000).

Introduction

Arsenic is a compound that is very toxic to many life forms. However, when arsenic is in its insoluble form, it is not a harmful compound. Arsenic in its insoluble form is found in minerals, such as arsenopyrite (FeAsS) (Santini, Sly, Schnagl, and Macy, 2000). The oxidation of the insoluble form of arsenic results in its transformation. Chemical and microbial processes often facilitate this oxidation. For instance, microbial processes can convert insoluble arsenic to arsenite (As III) (H_3AsO_3), now a soluble form of arsenic (Santini, Sly, Schnagl, and Macy, 2000). Acid mines often possess high concentrations of arsenite. The transformation of arsenite could be further oxidized by other microbial and chemical reactions. The oxidation of arsenite results in the formation of arsenate (As V ($H_2\ As0_4^- + H^+$). Arsenite and arsenate are both very toxic forms of arsenic. Arsenite is more toxic than arsenate (Santini, Sly, Schnagl, and Macy,

2000). This information is valuable to the advancement and improvement of bioremediation techniques.

History shows that arsenite-oxidizing bacteria was first identified in 1918 (Santini, Sly, Schnagl, and Macy, 2000). Research also shows that more of these organisms have been identified since 1918. Arsenite-oxidizing bacteria are heterotrophic, meaning an organism requires organic matter for growth. *Alcaligenes faecalis* is the most common arsenite-oxidizing bacteria. However, arsenite oxidizing bacteria's heterotrophic condition is considered a detoxification mechanism rather than a mechanism that supports growth.

Pseudomonas arsenitoxidans is the only organism known to gain energy in the presence of arsenite. This organism holds its ability to grow chemolithoautotrophically with arsenite, oxygen, and carbon dioxide. This experiment reports a new bacterium that was isolated from a gold mine in the northern territory of Australia that can also grow chemolithoautotrophically with arsenite. In addition, this organism can serve as an electron donor, with oxygen being an electron acceptor, and carbon dioxide (CO_2) or bicarbonate (HCO_3^-) as the carbon source (Santini, Sly, Schnagl, and Macy, 2000).

This discovery is important because it can help those interested in the bioremediation of arsenic understand the factors that affect remediating arsenic. Studies show that microorganisms can contribute to the toxification of arsenic. A site filled with arsenite-oxidizing bacteria will create more toxic forms of arsenic and increase the ineffectiveness of bioremediating arsenic (Santini, Sly, Schnagl, and Macy, 2000). Until the discovery of an organism or procedure that can counteract arsenic transformation, bioremediation of arsenic will remain ineffective and costly.

Materials and Methods

Samples of arsenopyrite were extracted from a gold mine in the Northern Territory of Australia in order to isolate arsenite oxidizers. (Santini, Sly, Schnagl, and Macy, 2000). Small pieces of rock were placed in bottles containing a minimal enrichment medium with arsenite absent. The bottles were sent to a laboratory and incubated for 7 days at 28ºC (Santini, Sly, Schnagl, and Macy, 2000). The enrichments were sub-cultured twice into an enrichment medium containing 10mm of arsenite (Santini, Sly, Schnagl, and Macy, 2000).

Growth of Pseudomonas arsenitoxidans

The *Pseudomonas arsenitoxidans* (NT-26) was grown in a growth medium consisting of 5mm of arsenite. Cultures were grown overnight under experimental medium (200 mL). Portions of the samples were centrifuged (15,000 x g, 10 minutes) in a microfuge to remove cells and the supernatant was frozen until analysis was performed (Santini, Sly, Schnagl, and Macy, 2000).

Analytical Methods of Arsenite

The concentration of arsenite was determined using a Varian spectra AA20 atomic absorption spectrophotometer. Arsenate was analyzed using a high-pressure liquid chromatography (HPLC) (Santini, Sly, Schnagl, and Macy, 2000).

Results and Conclusion

It was discovered that *Pseudomonas arsenitoxidans* NT-26 grew chemolithoautrophically (Santini, Sly, Schnagl, and Macy, 2000). *Pseudomonas arsenitoxidans* NT-26 is a gram-negative motile rod with two subterminal flagella (Santini, Sly, Schnagl, and Macy, 2000). This organism

gains energy for growth from arsenite oxidation. Also, this organism is able to grow in the presence of organic matter and oxidized arsenite throughout the growth process (Santini, Sly, Schnagl, and Macy, 2000). In addition, the growth of this organism continues to thrive in the presence of different yeast extract. It also was observed that the amount of growth was greater in the presence of arsenite, which suggests that the organism is gaining energy from the oxidation reaction and from the oxidation of yeast extract (Santini, Sly, Schnagl, and Macy, 2000).

From these results it can be concluded that *Pseudomonas arsenitoxidans* NT-26 is an arsenite-oxidizing bacterium that gains energy from arsenite oxidation. Pseudomonas ardenitoxidans NT-26 is a unique organism because it has the ability to grow rapidly with arsenite chemolitho-autotrophically. This discovery is significant because many arsenic oxidizing organisms have been discovered, such as *Bacillus arsenoxydans* and *Alcaligenes Faecalis*, but none of these organisms have the ability to grow chemolithoautotrophically. Therefore, the discovery of this bacteria species is an advancement in the understanding of microbial-arsenic interactions. The more knowledge acquired about microbial-arsenic interactions, the more researchers will begin to understand the benefits and limitations of bioremediating arsenic.

Summary

Arsenic is found in many parts of the environment and is toxic to the environment. It is the number one contaminant found in Superfund, Department of Defense, and Department of Energy sites. Exposure to arsenic can inhibit mitochondria functions and decrease the cellular production of ATP. Fever, anorexia, melanosis, and cardiac arrhythmia are caused by arsenic exposure. Acute exposure results in sensory loss in the peripheral nervous system. Chronic exposure can produce changes in the skin epithelium. This toxic element is a public health concern and will remain a health concern if an effective and innovative way to remove or reduce this toxic compound in soils, sediments, and groundwater sources is not found.

Arsenic is located in Group V of the periodic table. It is considered a semi-metallic compound. It exists in four oxidation states –3, 0, +3, +5. Arsenic's oxidation and environmental conditions affects its mobility and immobility conditions. Under aerobic conditions, arsenate (H_3As0_4, $H_2As0_4^-$, $HAs0_4^{2-}$, $As0_4^{3-}$) precipitates when metal cations are present.

Arsenic (As (V) 0) possesses the ability to co-precipitate iron oxyhydroxides under acidic and moderately reducing conditions. Arsenic mobility is increased when pH increases. However, arsenic compounds absorb in the soil and can travel short distances in ground and surface water. Arsenite co-precipitates with metal sulfides and has a high attraction to other sulfur compounds. This fact suggests that arsenic is capable of transforming to an immobilized precipitate form. As stated previously, as pH increases, arsenic's mobility is increased. This provides a limitation to the bioremediation of arsenic because once the compound is immobilized; variations in pH can reverse arsenic's immobility to mobility.

The case study *Mobilization of Arsenite by Dissimilatory Reduction of Absorbed Arsenate* discusses microbes that can mobilize arsenite. This experiment discovered that Fe (III)-respiring bacterium mobilizes arsenic. The bacterial strains *Sulfurospirillum arsenophilum* and *Sulfurospirillum barnessii* both can release arsenic into the environment. This information provides knowledge about what organisms mobilize arsenic. This suggests that microorganisms can be responsible for the spreading of arsenic in groundwater and soil sources. This also

confirms that microorganisms hold the ability to transform arsenic As (V) to As (III). This transformation is not an effective approach to environmental detoxification.

The case study, *A New Chemolithoautotrophic Arsenite-Oxidizing Bacterium Isolated from a Gold Mine: Phylogenetic, Physiological, and Preliminary Biochemical Studies*, describes the discovery of arsenite-oxidizing bacteria that can gain energy in the presence of arsenite. Pseudomonas arsentioxidans is the only organism identified to gain energy in the presence of arsenite. This organism is capable of growing chemolithoautrophically with arsenite, oxygen and carbon dioxide. This is a unique organism because most arsenite-oxidizing bacterial are heterotrophic, not chemolithoautotrophic. This discovery emphasizes the need to understand arsenic microbe-metal interactions. Microorganisms can contribute to the toxification of arsenic, thus limiting the effectiveness of bioremediation. This case study also discussed the techniques used to extract arsenic from contaminated soil and sediments. As stated earlier, the reduction of arsenate As (V) to As (III) is more toxic and soluble than As (V) (Lovely, 2001). Recent studies suggest that the As (V) reducing microorganism, *Sulfurospirillum barnessi,* has the capability to reduce and mobilize arsenate As (V), which was co-precipitated on ferrihydrite.

In addition to metal reduction, the production of organic acids by heterotrophic organisms can aid with the extraction of arsenic. Moreover, research is studying the ability of filamentous fungi and yeasts to volatilize arsenic. Documents show that several fungi species, such as *Gliocladium*, *Candida humicola*, and *Penicillium* species can convert monomethylarsonic acid to trimethylarsine, different forms of arsenic (Lovely, 2001). This process is described as arsenic methylation, which involves the transfer of methyl by S-adenosylmethionine (Lovely, 2001).

The significance of this study is the acknowledgement that fungi species can help remove or leach metals from industrial wastes. Overall, the bioremediation of arsenic involves reduction and oxidation of arsenic with the use of organisms. Based on these findings, the reduction and oxidation of arsenic is not an effective detoxification method because all arsenic forms are toxic. However, the use of fungi species may offer a way to leach arsenic from industrial waste sites. The production of organic acids with the use of heterotrophic organisms and the generation of sulphuric acid with the use of microorganism, such as *Thiobacillus*, offers some promising approach to the extraction of arsenic.

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CHROMIUM

Health and Toxicology

Chromium is found in rocks, animals, plants, and soil. The most common forms of chromium are Cr (II), Cr (III), and Cr (VI). Steel is made from chromium (0). Cr (III) appears naturally in the environment. Industrial processes produce chromium (II) and chromium (VI). The hexavalent chromium compounds (chromium VI) are industrial produced by the oxidation of chromium (II) compounds (ATSDR, 1999). The toxicokinetics of a given chromium compound depends on the valence state of the chromium compound (ATSDR, 1999).

Adsorption from Cr (VI) compounds is higher than Cr (III) compounds because chromate anion $(CrO_4)^{2-}$ enters the cells by facilitated diffusion (ATSDR, 1999). By passive diffusion and phagocytosis, adsorption of chromium III atoms can take place (ATSDR, 1999). Oral exposure to chromium occurs when it is inhaled; adsorption occurs in the lung and transfers across the cell membranes and into the gastrointestinal tract (ATSDR, 1999). Adsorption that occurs through oral exposure in humans varies because chromium particles vary in solubility (ATSDR, 1999). Dermal adsorption depends on physical and chemical properties of the compound, the fomite, and the skin type. Solutions that are concentrated with chromium (VI) compounds, such as potassium chromate, cause chemical burns (ATSDR, 1999).

Once chromium enters the blood stream, chromium compounds can be distributed to all organs of the body. Cr (VI) is unstable in the body and is reduced ultimately to Cr (III) by many substances like ascorbate and glutathione (ATSDR, 1999). Once this reduction occurs, excretion can occur through urine, hair, and nails. However, hair and nails provide minor pathways of excretion (ATSDR, 1999). Studies suggest that toxicity effects of Cr (VI) compounds result from the destruction of cellular components. Destruction of cells is caused by generation of free radicals. Chromium is a compound that should be dealt with caution because of its toxic effects.

Chemical and Physical Characteristics

Chromium only exists naturally on earth in its compound form, not in its elementary form (Evanko and Dzombak, 1997). Cr (VI) is the form of chromium that is mostly found at contaminated sites. This compound is the dominant form of chromium and is found in shallow aquifers, an aerobic environment. Cr (VI) can be reduced to Cr (III) by organic matter and components like S2- and Fe 2+ under anaerobic conditions. Anaerobic conditions often exist in groundwater (Evanko and Dzombak, 1997). Precipitation in the presence of metal cations, like lead, usually exists with chromium (VI) species like chromate ($Cr0_4^{2-}$) and dichromate ($CrII0_7^{2-}$). Adsorption on soil surfaces composed of iron and aluminum oxides usually occurs with chromate and dichromate. At a low pH (<4) Cr (III) is the dominant form of chromium. Cr (III) forms solution complexes with NH_3, OH^-, Cl^-, F^-, CN^-, $S0_4^{2-}$ and with soluble organic ligands (Evanko and Dzombak, 1997).

Chromium mobility depends on the sorption characteristics of the soil and the amount of organic matter present. Cr (VI) is more toxic and more mobile than all the forms of chromium. Chromium (III) is also mobile, but it decreases with adsorption of clays and oxide minerals below a pH of 5 (Evanko and Dzombak, 1997). Chromium can be transported via surface runoffs to surface waters while it is soluble or when it in its precipitated form. The increase in soil pH increases the leachability of Cr (VI). Usually when chromium is released into natural waters it is

deposited into the sediment where bioremediation of this metal can occur. (Evanko and Dzombak, 1997)

Case Study: Reduction and Precipitation of Chromate by Mixed Culture Sulphate-Reducing Bacterial Biofilms

Introduction

Industries such as leather tanning, petroleum refining, and pulp production generate large quantities of chromium in the environment. The increase in chromium pollution has been recognized and has caused a demand to produce chemical and biological remediation techniques (Smith and Gadd, 2000).

Recent studies have shown that microorganisms are capable of altering the redox state of toxic metals, organometals, and radionuclide contaminants. Chromium is a metal compound that possesses a high oxidation state and high solubility. Chromium has many oxidation states, from -2 to +6. These characteristics make the remediation process of chromium difficult (Smith and Gadd, 2000).

It is now recognized that sulphate-reducing bacteria are obligate anaerobic heterotrophs and can cause toxic metals to precipitate to insoluble metal sulphides. It is also known that biofilms can affect the sorption, transportation, and decomposition of metal and radionuclide pollutant species (Smith and Gadd, 2000). These techniques are used to remediate chromium despite their challenges.

The purpose of this experiment is to determine the ability of sulphate-reducing bacterial biofilms to reduce hexavalent chromium and to analyze the effects of Cr (VI) on sulphate (Smith and Gadd, 2000).

Materials and Methods

Sulphate-reducing Bacteria

The mixed culture of sulphate-reducing bacteria was used as the organism for the experiment. The biofilm cultures were sub-cultured at 14-day intervals (Smith and Gadd, 2000).

Biocell

The biocell consisted of a 13x20 mm Perspex anaerobic system with an inflow and outflow containing 7x14 cm Plastikard polystyrene sheeting (Smith and Gadd, 2000).

Chromium

The chromium assay was analyzed using a Pye Unicam SP9 atomic absorption spectrophotometer (Smith and Gadd, 2000).

Sulphate

The sulphate in this experiment was assayed by a Metrohm 690 ion chromatograph with a 697 IC pump, and a 750 auto sampler with PRP-X100 column (Smith and Gadd, 2000).

Sulphide
The sulphide was assayed by DC polarography using a Metroham 663 VA polarograph stand, and Polarecord recorder (Smith and Gadd, 2000).

Protein
The bacterial biofilm was analyzed by measuring the protein with the Bradford method (Smith and Gadd, 2000).

Results and Conclusion
This experiment demonstrated that Cr (VI) is converted to an insoluble, less toxic trivalent form with the use of mixed culture of sulphate-reducing bacterial biofilms. In basic solution, chromium forms a yellow chromate ion (CrO_4^{2-}). Therefore the reduction of chromate causes a decrease in the yellow chromate ion. It was noted only a small percentage of chromium remained in solution Cr (VI) at 48 hours (Smith and Gadd, 2000). The mass balance studies showed that the majority of the chromate was transformed to an insoluble form, which accumulated at the base of the biofilm. The bacterial biofilm did not retain a lot of the chromium. In this study, the mixed culture sulphate reducing bacterial biofilms were capable of reducing 88% of the total hexavalent chromium over a 48-hour time period. This experiment suggests that the biological mechanism of Cr (VI) reduction is similar to that found in other sulphate-reducing bacterial biofilms (Smith and Gadd, 2000).

Case Study: Bioremediation of chromium with Cheese Whey (Rynk, 2004)

Introduction
The city of Emeryville is concerned about the future use of its groundwater and the health risks of construction workers who are responsible for removing soil from a local site (Rynk, 2004). The new owner of the site obtained a loan from Emeryville under the city's Capital Incentives for Emeryville's Redevelopment and Remediation program to conduct an engineering evaluation/cost analysis to determine remediation options for groundwater contaminated with hexavalent chromium. Three remediation approaches were evaluated: natural attenuation, excavation followed by pump-and-treat, and bioremediation using cheese whey.

Upon evaluation, it was determined that natural attenuation of hexavalent chromium would be inefficient and timely, and excavation followed by pump-and-treat, which would involve removing and landfilling the hexavalent chromium-contaminated soil and pumping and treating the contaminated groundwater plume, would eventually result in contamination of the waste stream (Rynk, 2004). However, results of a pilot test involving the injection of cheese whey into the soil and groundwater showed that this method would be the most effective and cost efficient alternative. The use of cheese whey is an attempt to use an inexpensive nutrient supply to nourish microorganisms that possess the ability to convert hexavalent chromium to trivalent chromium (Rynk, 2004). The pilot test produced a 99% decrease of hexavalent chromium in several of the wells over a 2-month period (Rynk, 2004).

Site Description
The site is in a commercial/industrial land located in Emeryville, California, which is located approximately ten miles northeast from San Francisco (Rynk, 2004). This site operated as a chrome-plating facility from 1951 through 1967 (Rynk, 2004).

Bioremediation Process

The full-scale remediation of the site was scheduled to begin in early 2004 and involved monthly injection of diluted cheese in 22 injection wells. Fifteen thousand gallons of cheese whey was required to conduct the project (Rynk, 2004). Each monthly injection consisted of diluting the cheese whey with water. The dilution and injection was conducted using portable injection trucks. After the dilution process, 300 gallons of diluted whey solution was injected into each of the 22 injection wells. Each monthly injection took eight days to complete (Rynk, 2004).

Monitoring of the Chromium

In order to monitor the effectiveness of the remediation, the groundwater was monitored for total hexavalent chromium and other chemical indicators, such as pH, oxygen-reduction, dissolved methane, and oxygen (Rynk, 2004). The monitoring wells were placed around the plumes. (Rynk, 2004).

Conclusion

Studies show that in situ groundwater remediation can increase growth of microorganisms and provide effective treatment. The organic matter used during the remediation process depleted the oxygen levels and thus shifted the environment to an anaerobic atmosphere. Furthermore, the organic compounds can serve as an electron donor for a chemical reduction process (Rynk, 2004). This project demonstrated that cheese whey is an important component to the remediation of hexavalent chromium.

Summary

Chromium is a compound that is found in nature. The common forms of chromium are Cr (II), Cr (III), Cr (VI). This section of the paper discussed how the chemical and physical properties of chromium contribute to health effects it causes and the techniques used to bioremediate this compound.

In regards to health effects, Cr (VI) is the main contributor to generating health problems. Inhalation exposure to Cr (VI) begins with the lungs and spreads across cell membranes. Cr (VI) can also gain entry to the body by dermal exposure. For example, solutions concentrated with potassium chromate can causes chemical burns. These burns provide a dermal pathway. Studies support that toxicity effects of Cr (VI) result in the destruction of cellular components. It is important to study chemical and physical characteristics of chromium to understand its toxicity effects.

This section also discussed the effects valence has on the mobility and immobility of chromium. Chromium (VI) is more toxic and more mobile than all other forms of chromium. Cr (III) is less mobile, especially in the presence of clays and oxide minerals below a pH of 5. Studies consistently show that organic matter, pH of sediments affect the mobility of chromium. Organic matter and pH can convert Cr (VI) to Cr (III), causing a decrease in mobility. Microorganisms and other environmental factors, such as pH, climate, and presence of minerals, contribute to the immobility or mobility of chromium.

The case study "*Reduction and Precipitation of Chromate by Mixed Culture Sulphate-Reducing Bacterial Biofilms*" further discusses the effects microorganisms have on chromate. Recent studies have shown microorganisms are capable of altering the redox state of toxic metals,

organometals, and radionuclide contaminants. Chromium is a compound that possesses a high oxidation state and high solubility. Chromium has many oxidation states from -2 to +6. These characteristics create a challenge for the bioremediation of chromium.

This experiment discovered that Cr (VI) is converted to an insoluble, less toxic Cr (III) with the use of sulphate-reducing bacterial biofilms. In this study, the mixed culture, sulphate-reducing bacterial biofilms, reduced 88% of the total hexavalent chromium over a 48-hour time period. Based on this study, it is rational to conclude the use of microorganisms is a way to immobilize and detoxify compounds, such as chromium.

"*Bioremediation of Chromium with Cheese Whey*" featured a case study in Emeryville, California, where cheese whey was used to treat groundwater contaminated with Cr (VI). Each month, cheese whey was injected into 22 injection wells. These wells were monitored with wells located around the plumes to determine the effectiveness of the remediation. This project emphasizes the importance of microorganisms to the detoxification of chromium.

Overall, experiments and literature suggest that biofilms and strains of *Pseudomonas putida* assist with the reduction process of chromium. The application of these organisms is mostly done in the labs. This means that the bioremediation of chromium is still at lab scale because more research needs to be done on the preventing leaching of this contaminant. The microbial and chromium interaction is still not completely understood; until it is understood, researchers will remain skeptical of using bioremediation to remediated chromium.

Further Remediation Techniques for Chromium

Studies show new bacterial strains extracted from contaminated sites in Pakistan hold the ability to reduce toxic Cr (VI) to Cr (III) (Lloyd and Lovely, 2001). Biofilms consisting of sulfate-reducing bacteria also hold the ability to reduce and precipitate Cr (VI). Research also shows that microbial consortium is able to couple oxidation of phenol to Cr (VI) (Lloyd and Lovely, 2001). Purification of chromate reductase can occur with the use of bacteria strain Pseudomonas putida (Lloyd and Lovely, 2001). Remediation of chromium is moving toward discovering more bacteria strains that can reduce Cr (VI).

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LEAD

Health and Toxicology

Lead is commonly found in industrial settings and lead exposure has the tendency to cause adverse health effects. The adverse health effects induced by lead exposure are dependent on two important components: dose (how much of a contaminant) and duration (how long there has been contact with the contaminant) (ATSDR, 1999). Exposure to lead can occur from eating and drinking contaminated water or breathing in high levels of lead (ATSDR, 1999). Children are exposed to lead through eating lead based paint chips or through skin absorption while playing in lead contaminated soil. This hazardous material enters the air we breathe, the water we drink, and the plants and animals we consume.

Lead is a health problem for communities that reside near acidic water supplies. Acidic water can cause lead to leach into our drinking water supply from lead pipes, leaded solder, and brass faucets. The EPA reports that more than 99% of the public's drinking water contains less than 0.05 parts per million of lead, which is the maximum contaminant level for lead (ATSDR, 1999).

Refining facilities, brass foundries, rubber refineries, and steel welding operations are settings in which exposure to lead to optimal. The Agency for Toxic Substances and Disease Registry (ATSDR) states between 0.5 and 1.5 million workers are exposed to lead at the work place. More than 200,000 workers in California alone are exposed to lead (ATSDR, 1999). This suggests that many people, especially blue-collar workers, are disproportionately exposed to lead and suffer adversely from health problems because of it.

Once lead has entered the blood stream, it flows to various parts of the body. Lead most commonly enters the body through ingestion, and then travels to the lungs then swiftly through the blood stream to other parts of the body. Lead does not change form once it enters the body. Lead in the blood stream travels to the soft tissues of the body, such as the liver, kidneys, lungs, brain, spleen, muscles, and heart. Over time, lead particles have the ability to move into the bones and teeth. Lead impairs communication between cells and modification of neuronal circuitry (Curtis D. Klassmen and John B. Watkins 111, 2003). ATSDR reports that 94% of the total amount of lead in the body is contained in bones and teeth. For children, 73% of lead in their body is stored in their bones (ATSDR, 1999). Lead that enters the bones can remain there for decades. However, over time, lead that is stored in the bones can reenter the blood stream and organs.

Excretion of lead occurs through urine and feces when it is not stored in the bones. ATSDR reports that 99% of the amount of lead absorbed in an adult's body will leave the body through waste within a couple of weeks. In contrast, only 32% of the lead absorbed by children's bodies leaves as waste. Means of exposure determine if the lead entering the body will be removed or accumulate in the body tissues, especially within the bone (ATSDR, 1999).

Lead toxicity usually begins with the nervous system in both adults and children. Exposure to lead can cause weakness in fingers, wrists, or ankles. Studies show long-term exposure to lead at work can lead to decreased performance. High levels of lead can cause damage to the brain, kidney, and can cause anemia in both adults and children (ATSDR, 1999).

Lead produces a range of different effects that vary in adults and children. A child that ingests large amounts of lead may develop blood anemia, kidney damage, colic, muscle weakness, and brain damage. These combined health effects can cause death in children. However, low levels of lead contamination can also cause detrimental effects to the blood and brain of a child. Pregnant women can experience a miscarriage if exposed to high levels of lead during there pregnancy (ATSDR, 1999). High levels of lead can also damage organs that are responsible for sperm production. Consistent and long exposures to lead can potentially produce noxious health effects.

Chemical and Physical Characteristics

It is essential to understand the chemical and physical characteristics and processes of a metal to understand its full bioremediation potential. The mobility and immobility of compounds is dependent on their physical and chemical characteristics. These two characteristics dictate a metal's particular mechanism.

Different conditions allows for lead to reside in different forms. In nature, lead exists with an oxidation state of 0 or +II. Lead possesses a molecular weight of 207.20 and is a bluish-gray colored solid. It has a melting point of 327.4 and a boiling point of 1,740 ºC. Lead (Pb^{2+}) and lead hydroxy compounds are the most stable form of the lead. However, Pb^{2+} is the most reactive and common form of lead. This form produces mononuclear and polynuclear oxides and hydroxides (Evanko and Dzombak, 1997).

In the presence of inorganic compounds (such as Cl, CO_3^{2-}, SO_3^{-}, PO_4^{3-}) and organic ligands (such as humic, folic acids, EDTA) low soluble lead compounds form. The concentration of dissolved salts, pH, and minerals affect the amounts of lead found in surface water and groundwater. In surface water and groundwater systems, lead can appear in its precipitate form ($PbCO_3$, Pb_2O, Pb(OH)2, $PbSO_4$) and is capable of absorbing into the surface of minerals (Evanko and Dzombak, 1997). Adsorption, ion exchange, precipitation, and complexation with organic matter are mechanisms that limit the amount of lead leaching through surface water or groundwater. Tetramethyl lead, the volatile organolead compound, forms in anaerobic sediments by an alkyllation mechanism (Evanko and Dzombak, 1997). Volatile lead creates unsafe conditions for water supplies because leaching is prominent in this form. In most cases, a precipitated form of lead is the more desirable form because the lead is immobilized.

Subsurface Mobility and Immobility

In situ bioremediation is the application of remediation, which occurs below the ground or at the site. Subsurface bioremediation refers to remediation that occurs above the groundwater source. The subsurface of the earth is composed of a vapor phase and a capillary fringe above the ground surface. Subsurface bioremediation usually involves the remediation of unsaturated soil or soil. Bioventing, a process that requires a supply of air or oxygen to soil to stimulate aerobic biodegradation of contaminants, is an example of subsurface bioremediation. Components like adsorption, ion exchange, precipitation, and complexation with organic matter are factors that contribute to the high adsorption rate of lead in soil (Evanko and Dzombak, 1997). These components also contribute to the immobilization of lead on surface water or groundwater.

Groundwater Bioremediation

Groundwater is water that is located below the earth's subsurface. Groundwater is usually located near the residual saturation point. Groundwater bioremediation can either occur in situ or ex situ.

Aerobic and anaerobic reactions are also examples of in situ groundwater processes. An aerobic process involves air or oxygen placed into the groundwater in order to promote biodegradation of contaminants (USEPA Office of Solid Waste and Emergency Response, 2001). Anaerobic process consists of a carbon source such as molasses, lactic acid, or hydrogen release compounds are injected into groundwater to enhance biodegradation of contaminants (USEPA Office of Solid Waste and Emergency Response, 2000).

Case Study "Dynamics of Lead Immobilization in Sulfate Reducing Biofilms" (Beyenal and Lewandowski, 2004)

Introduction

The purpose of this study is to examine the effects that minerals (specifically quartz and hematite) have on a biofilm consisting of *Desulfovibro desulfuricans* (Beyenal and Lewandowski, 2004). Quartz and hematite were selected to produce mineral-metal interactions. Studies support that under anaerobic conditions the bacterium *Desulfovibrio desulfuricans* is capable of immobilizing lead. In this experiment, the minerals released iron ions. Iron (Fe III) can be reduced by sulfate-reducing bacteria (Beyenal and Lewandowski, 2004).

Although the transformation of Fe (III) to Fe (II) was not quantified in this article, the chemical process where sulfate-reducing bacterial biofilm removed lead was observed. Sulfate-reducing bacteria generated energy by the oxidation of organic compounds and the reduction of sulfate ions (Beyenal and Lewandowski, 2004). This chemical process produces H2S and then dissociates into bisulfide. Since lead is a component of this experiment, lead ions were present during the chemical reaction. The lead ions in the presence of bisulfide caused the bisulfide to precipitate lead ions as lead sulfide (PbS). Minerals were also in the presence in the process described above. The minerals found near the metal ions served as nourishment for the biofilm (Beyenal and Lewandowski, 2004). Another component of this reaction is oxygen. It was realized by the researchers that once metal sulfide is immobilized, the introduction of oxygen would potentially cause remobilization of the metal hydroxides, oxides, and sulfates. These factors demonstrated that the stability of lead is affected by the composition of the biofilm, the minerals present, and the presence of oxidants (Beyenal and Lewandowski, 2004). In summary, this experiment attempted to bring understanding to the dynamics of biosorption of lead, which involves the passive accumulation of metals or radioactive material by biological organisms (Lovely, 2001).

Materials and Methods

Organism

Desulfovibrio desulfuricans was used to grow the sulfate-reducing bacterial biofilm. The experiment consisted of *D. desulfuricans* batch cultures placed in 100 mL serum bottles for three days. The reactors were inoculated with 20 mL of *D. desulfuricans* (Beyenal and Lewandowski, 2004).

Sulfate-Reducing Biofilms
The sulfate-reducing biofilms came from a pure culture of *D. desulfuricans* (Beyenal and Lewandowski, 2004). Batch cultures were created by growing *D. desulfuricans* in 100 mL serum bottles for 3 days (Beyenal and Lewandowski, 2004).

Growth of Biofilm
The biofilm was grown using a metal-toxicity medium. This metal-toxicity medium reduced the lead complexation and precipitation. Once the metal-toxicity medium was prepared, the solution was adjusted to 7.2 using 1N HCL 0.1 g ascorbic acid, and 0.1 g sodium thioglycollate was added to a liter of growth medium (Beyenal and Lewandowski, 2004). This mixture was designed to stimulate the growth of the sulfate-reducing bacteria.

Reactors
The reactors consisted of two flat plates; one filled with hematite and the other with quartz. The reactors had a dimension of 3.5 cm deep, 2.5 cm wide, and 34 cm long with a total working volume of 120 mL. The quartz chips were 2.5x7.5x 0.1 cm3 and the hematite chips were 0.5 x0.5 cm3 (Beyenal and Lewandowski, 2004). The hematite chips were cleaned with acetone and rinsed twice with distilled water and polished with sandpaper. After the minerals were cleaned, they were placed at the bottom of the reactors. This concluded the preparation of the reactor; analysis of the minerals proceeded after the preparation.

The minerals were analyzed with the use of an X-ray diffraction tool. This x-ray diffraction tool determined that the hematite chips were 90% hematite and 10% magnetite and contained traces of cristobalite minerals (Beta-SiO2) (Beyenal and Lewandowski, 2004).

Quantifying Lead and Iron Ions
In order to determine the total lead and iron concentrations in the biofilms, the biofilm was removed from the chip and placed in distilled water for two minutes. It was then treated with three-N nitric acid. Then nitric acid (HNO_3) was used to dissolve the precipitated metals (Beyenal and Lewandowski, 2004). Lastly, the samples were centrifuged for 20 minutes at 6000 rpm. After centrifugation, the pellet was discarded and the supernatant was used for iron and lead analysis.

Results and Discussion
The data from this experiment demonstrate that lead was completely immobilized with the biofilm grown on the quartz. However, the biofilm grown on hematite did not immobilize all of the lead (Beyenal and Lewandowski, 2004). In addition, when the biofilms were exposed to atmospheric oxygen, the rate of lead immobilization decreased.

Biofilms grown on the hematite and on the quartz were heterogeneous because they were composed of cell clusters (Beyenal and Lewandowski, 2004). The average hydrogen sulfide concentration was higher in the biofilms grown on quartz than in the biofilms grown on hematite. Furthermore, during the 18 weeks of operation, iron was continuously being released from the hematite. Lead precipitated more from the biofilms grown on quartz, which may suggest that microorganisms present on some minerals, like quartz, allow for efficient precipitation of lead. The results also suggest that metals released from redox-sensitive minerals can precipitate in the presence of microbial-generated hydrogen sulfide and decrease the metal-binding capacity of the contaminant metal (Beyenal and Lewandowski, 2004).

Conclusion

The results show that the maximum efficiency of metal immobilization in sulfate-reducing biofilms can be accomplished when minerals are redox-insensitive and located in the earth's subsurface (Beyenal and Lewandowski, 2004). Final results suggest that metals released from redox-sensitive minerals can precipitate in the presence of hydrogen sulfide and then decrease the overall metal-binding capacity of the system. This process, which involves hydrogen sulfide binding to metals released from minerals, occurs because of the biofilm's heterogeneity and kinetic limitations.

This research demonstrates that lead precipitates more in the presence of biofilm that is located near redox-insensitive compounds, such as quartz. Quartz does not release many metal ions. As previously mentioned, metal ions released from a mineral can decrease the metal-binding capacity of the compound.

Biofilm grown on hematite can cause a decrease in lead precipitation. The minerals also contribute to the amount of hydrogen sulfide released. According to the microelectrodes, the biofilm located on quartz produced higher concentrations of hydrogen sulfide (H2S), unlike the hematite that generated smaller concentrations of hydrogen sulfide. The concentration of hydrogen sulfide affected the growth level of the biofilm, which affected the lead's binding capacity. The results obtained from this experiment demonstrate the effects of sulfate-reducing biofilms in the presence of minerals on lead immobilization.

Case Study: Metal Bioremediation Through Growing Cells (Malik, 2003)

Introduction

This journal article, written by Anushree Malik, describes the beneficial use of bacterial and fungal strains in removing metal pollution. Studies show that bacteria, yeast, and fungi specimens have the capability of extracting metal contaminants from sites that are saturated with metals like cadmium, copper, nickel, cobalt, zinc, and lead. Industries are seeking low-cost and efficient methods of removing metals from contaminated sites.

Industries use physico-chemical processes, such as oxidation and reduction, chemical precipitation, filtration, electro-chemical treatment, evaporation, ion exchange, and reverse osmosis, to remove lead. However, these processes are limited because some sites contain contaminants that are not affected by these processes. Studies show that contaminating reagents cause desorption, which in turn causes toxic sludge and secondary environmental pollution (Malik, 2003). These limitations further explain the extensive search to find more cost-effective processes to remove metal pollution.

Biotechnology provides a cost-efficient way to remove hazardous material, and can be effective at selectively removing metals. Biotechnology involves the use of organisms to respond to pollution through techniques like biosorption, precipitation, complexation, and oxidation-reduction reactions.

Biosorption, the passive uptake of heavy metal ions, involves the use of bacterial compounds to remove abstract metals or radioactive elements from contaminated sources. Studies show that heavy metals can be biosorbed into binding cites present in cellular structures. Biosorption alone

is not sufficient for effective metal remediation. Growing cells have unlimited capacity to bind to organo-metallic complexes, degrade organic compounds, and take up other inorganic ions such as ammonium (Malik, 2003). This study shows that biosorption accompanied with active and growing cells may provide a better way of removing metals.

Precipitation involves metal cations and anions (e.g., $S0_4^{2-}$, S^{2-}, oxalate, HPO_4^{2-}) forming insoluble aggregates (salts or complexes), such as sulfides, carbonates, oxides, and oxalates. Complexation involves metabolites and siderophores that cause metal mobilization (Malik, 2003). Oxidation and reduction are chemical reactions that involve an organic contaminant that is oxidized (loses electrons), and a chemical or an organism that is reduced (gains an electron). The organic contaminant that is oxidized is called the electron donor and the chemical or organism that is reduced is called the electron acceptor. The energy gained from the electron donor is stored and used to produce more cells for the electron acceptor.

Results and Conclusion

The experimenter focused on where the bioaccumulation of metals occurs in living cells. The location and resistance mechanism is dependent on the strain. This experiment discovered that *Pseudomonas marginalis*, a bacterial strain, isolated from lead-contaminated soil produced a higher resistance and extracellular lead with high extracellular polysaccharide production. *Bacillus megaterium*, another bacteria strain, isolated from soil containing high soluble lead demonstrated lower resistance and intracellular accumulation of lead. This result confirmed that bacteria, fungi, and any active cell can react differently to hazardous materials. It also confirmed that the bioaccumulation of lead occurs in different portions of a cell.

Plectonema boryanum, an organism, confirmed that polyphosphate bodies absorb zinc, lead, manganese, and aluminum. Polyphosphate bodies are components of living cells that participate in the active uptake of metals. The experiment on this strain suggested that lead could be found in the cytoplasm of cells. When lead entered *Saccharomyces cerevisiae* cells, most of the lead was found in the cytoplasm in two hours. Observing the interaction of the lead and *Saccharomyces cerevisiae* and *Plectonema boryanum* explained the route of lead absorption. First, lead enters the cell and binds to the cell wall. Then, lead accumulates in the cell wall. Finally, it travels to the cytoplasm (Malik, 2003).

This experiment demonstrated that lipolysaccharides absorb metals like cadmium, copper, lead, and zinc. The organism *Bradyrhizobium joponicum* confirmed that a lipololysaccharide mutant, lacking o-polysaccharide, binds 50%-70% less than a non-mutant lipololysaccharide. This suggested that lipololysaccharide molecules *of B. japonicum* affect the precipitation of metals (Malik, 2003). Components of living cells affect the biosorption of metals.

This study also examined the bacterial stain *Desulfosporosinus orientis*, to identify its ability to biosorb metals. Contaminants, such as lead, cadmium, zinc, and copper, were found in groundwater located at a car battery recycling plant. Groundwater contamination often requires a different remediation approach than surface water. The experimenters discovered that *Desulfosporosinus orientis* assists with sulfate reduction and causes complete precipitation of metal sulfides. This discovery helps with the advancement of bioremediation of metals, such as lead, cadmium, zinc, and copper, located in groundwater sources.

Metal pollution endangers the vitality of the world's ecosystem. These organisms used in the experiment offer a promising resolution to preserving the ecosystem. Studies show that biosorption of metals with the use of dead biomass are not effective removal techniques for recalcitrant metals, such as nickel (Malik, 2003). Therefore, the use of lively metal-resistant bacterial/fungal strains may be a more effective way to remove metal contaminants. Active bacterial/fungal strains are able to function in extreme pH conditions and high metal concentrations, thus providing a practical and economical solution for the removal of metals (Malik, 2003).

Summary

Lead is a metal compound that can be found in all parts of the environment. Lead is a product of industrial activities like mining and manufacturing. Lead is a metal contaminant prevalent in Superfund, Department of Energy, and Department of Defense sites (Malik, 2003). It is a metal compound that needs to be removed as soon as possible from water and land sources around the United States.

Concern over lead's detrimental health effects contributes to research that concentrates on the bioremediation of lead. High levels of lead can cause damage to the brain, kidney, and anemia in both adults and children (Malik, 2003). Exposure to lead can cause premature births in pregnant women. Thus, the data show that lead is a contributor to health problems in the United States.

Studies like the *Dynamics of lead immobilization in sulfate reducing biofilms* help researchers understand how lead can be immobilized in groundwater. This journal article showed how minerals affect the precipitation of lead. Minerals like quartz and hematite affect the precipitation level of lead. Sulfate-reducing biofilms are also affected by minerals (Malik, 2003). Understanding the effects of minerals and biofilms can help others find effective ways of removing lead from contaminated sites.

The article *Metal bioremediation through growing cells* demonstrated that many bacterial and fungal strains can assist with lead removal. Organisms like *Pseudomonas marginalis*, *Plectonema boryanum*, and *Desulfosporiosinus orients* can all assist with lead uptake (Malik, 2003). This suggests that bioremediation using growing microbes is a practical alternative to biosorption removal of metal contaminants from industrial sites.

Research today is focusing on the precipitation of metal sulfides utilizing sulfate-reducing bacteria. The Mine Waste Technology Program of the United States Environmental Protection Agency and Department of Energy uses sulfate-reducing bacteria for bioremediation purposes. They also use passive treatment systems like compost for remediation. This is currently found not to be an effective treatment because the precipitates are not guaranteed to stay in their precipitated forms (Malik, 2003). Active treatment methods often use sulfate-reducing bacteria for the production of hydrogen sulfide, which causes rapid precipitation and recovery of metal sulfides (Beyenal and Lewandowski, 2004).

However, the challenge still exists to find fungal or bacteria biofilms that can work in many different conditions and keep the metals from leaching into the environment. It is important for researchers to discover ways to tackle this challenge; otherwise industries will continue to be skeptical about using bioremediation at metal-contaminated sites. (Beyenal and Lewandowski, 2004).

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MERCURY

Health and Toxicology

Metallic mercury has been found at 714 hazardous waste sites in the United States (ATSDR, 1999). The abundance of mercury present within these contaminated sites causes health and environmental problems to surrounding ecosystems. Chronic exposure to mercury can cause detrimental effects to human health and the environment. Exposure to mercury can come from a number of different routes, such as through dental amalgam fillings, household products, fluorescent light bulbs, broken thermometers, and industrial settings. Exposure to methyl mercury by these routes can cause health problems. A major source of exposure to methyl mercury comes from the consumption of fish. Methyl mercury is the most toxic species of mercury because it possesses a high adsorption rate to membranes. Neurotoxic health effects are common after exposure to methyl mercury. Pregnant women who consume large amounts of fish or who are exposed to high levels of mercury are at an increased risk of exposing their fetus to methyl mercury. Therefore hospitals and clinics advise pregnant women to limit the amount of fish they consume. Physical symptoms of methyl mercury exposure can be identified by paresthensia and numbness in the fingers and toes. Difficulty swallowing and clumsiness—called ataxia—are also symptoms of methyl mercury exposure (ATSDR, 1999). After ataxia sets in, neurasthenia can occur, followed by fatigue and an inability to concentrate. As methyl mercury continues to poison the body, tremors and vision and hearing loss develop. The final stage of methyl mercury poisoning is coma followed by death (ATSDR, 1999). Methyl mercury is a substance that should be addressed with caution and advisory because of its harmful health effects.

Mercury in the form of mercuric chloride is an example of a mercuric salt. Oral ingestion of mercuric salts is said to cause severe abdominal cramps and bloody diarrhea (Klaasen and Watkins III, 2003). These symptoms are usually accompanied by corrosive ulceration and necrosis of the gastrointestinal tract and renal failure within 24 hours of proximal tubular epithelium necrosis. Any great exposure to mercury leads to the intake of mercury by various organs of the body. Once mercury is absorbed into the body, its elemental or metallic form is oxidized forming divalent mercury. Mercury vapor absorbed through inhalation penetrates the body's red blood cells and is transformed to divalent mercury (Klaassen and Watkins III, 2003). A portion of inhaled mercury vapor in the form of metallic mercury is transported to more distal tissues where transformation may occur. Inhalation of mercury vapor at high levels may produce an acute, corrosive bronchitis and interstitial pneumonitis. With increased exposure to mercury, the body's system begins to show physical signs of effects. For example, the fingers begin to tremor followed by memory loss; severe depression becomes apparent after long exposure to mercury. Mercury toxicity can also cause severe salivation and gingivitis (Klaasen and Watkins III, 2003).

Chemical and Physical Characteristics

Mercury exists in several forms in the environment. These forms include metallic mercury, also known as elemental mercury, inorganic mercury, and organic mercury. Mercury is a unique and rare metal found in nature. It is found in a sulfide ore cinnabar in Spain, Russia, Mexico, Canada, and Algeria (Evanko and Dzombak, 1997). It is known for its high surface tension, flow behavior, and its electrical conductivity (NABIR, 2003). It is these characteristics that make mercury a desirable for use by industries. Mercury is used in dental products, thermometers, and neutron absorbers used by nuclear power plants (NABIR, 2003).

Department of Energy facilities serve as a source of mercury (NABIR, 2003). Mercury as an inorganic compound that can exist in three oxidation states (0 in its elemental state, +1 in its mercurial state, and +2 in its mercuric state). All three of these oxidation states of the inorganic mercury are hazardous. Inorganic mercury can be combined with other compounds, such as chlorine, sulfur, or oxygen forming white powders or crystals. These compounds can be used as fungicides or as elemental components of skin-lightening creams. Most inorganic mercury comes in the form of mercuric sulfide, which along with mercuric oxide is used as a coloring agent in tattoos and paints. Methyl mercury is the most common form of organic mercury. This form is most prevalent in freshwater and salt water. Another form of mercury is metallic mercury, which is used to produce chlorine gas, extract gold from ore, and make dental fillings. Metallic mercury is also used in religious practices in Latin America and Caribbean countries (ATSDR, 1999). Mercury can possess many forms and hold many industrial uses.

Any mercury transformation or immobilization process is dependent on pH. Organic or inorganic mercury can be reduced to elemental mercury under chemical reducing conditions. At a high pH, the absorption of mercury increases. Mercury can also participate in co-precipitation with sulfides, which aids in the removal of mercury from a solution. Mercury's (Hg^{2+}) ability to form strong bonds with many different inorganic and organic ligands suggests that it is very soluble in its oxidized form.

Demethylation of methyl mercury can further assist with the transformation of mercury. This process allows elemental mercury to be transformed under anaerobic conditions. Acidic conditions (pH< 4) favor the production of methyl mercury, where as higher pH values initiate precipitation of mercury sulfide HgS (s) (Evanko and Dzombak, 1997). Mercury transformation processes are important to the advancement and development of bioremediation of mercury located in groundwater and the subsurface of the earth.

Groundwater Treatment

Surface water treatment methods do not provide adequate treatment for the removal of heavy metal ions, especially for mercury that is found in groundwater (Vilensky, Berkowitz, and Warshawsky, 2002). Therefore the remediation of mercury contaminated groundwater requires a different approach than subsurface remediation.

Field Scale: Removal of Mercury from Chemical Wastewater by Microorganism in Technical Scale (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000).

Introduction

Mercury is a toxic element once it binds to sulfydryl groups of enzymes and proteins. The combination of mercury and sulfydryl groups can halt cell functions within an organism. This toxic combination is found in sediments. Aquatic organisms and other organisms are exposed to mercury when methyl mercury is present in the ground. Organisms that are exposed to mercury suffer from chronic diseases or death. Mercury is harmful to many living species; therefore the discharge of mercury needs to be prevented. Mercury-resistant bacteria offer a possible cost-effective method for removing mercury. This type of bacteria reduces soluble Hg (II) to insoluble metallic Hg (0) by means of cytoplasmic enzyme mercuric reductase, which is encoded by the gene, mer A. Reduced mercury changes to small droplets of metallic mercury. This is promising information for removing mercury from sites (Wagner-Dobler, Von Canstein, Li,

Timmis, and Deckwer, 2000). This field study attempts to remove mercury from chloralkli electrolysis wastewater and retain it within a bioreactor.

Material and Methods

Bacteria

Four subspecies of *Pseudomonas putida* (Spi3, Spi4, Kon12, Elb2), two subspecies of *Pseudomonas stutzeri* (Ibu3, Ibu 8), and one subspecies of *Pseudomonas fulva* (Spi11) were used as inoculant strains (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000). Single colonies were picked from plated precultures (500 mL) that were inoculated and grown for 48 hours at 30 ºC in rich liquid medium consisting of sucrose 10g/L, yeast extract 10 g/L, NaCl 30g/L, Hg (II) 5.0mg/L, sodium phosphate buffer 0.25 M, ph 7.0 (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000). Pre-cultures were transferred to seven, 15-liter fermentation vessels containing 3 L of rich liquid medium and antifoaming agent. A fed-batch fermentation was done for 4 days at 35 ºC. Cultivation of the inoculant strains stopped after four days. (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000).

Mercury Concentrations

Mercury concentrations were analyzed using two automated instruments from Mercury Instruments, a company located in Karlsfeld, Germany. The reduction of Hg (II) to Hg (0) was done by the chemical compound $SnCl_2$ (20 g/L) (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000). Metallic mercury was volatilized into the air; this volatilization was determined by spectroscopy at 253.7 nm.

Bioreactor

Mercury-resistant bacteria and wastewater was added to the bioreactor. The initial mercury outflow concentration was 900 µg/L, representing a retention efficiency of 82% at a mercury inflow concentration of 5 mg/L. This resulted in an average mercury outflow concentration of 306 µg/L for three hours, which represented a retention efficiency of 97%. After inoculation, full mercury removal was achieved without an adaptation period of the bioreactor to the on-site wastewater (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000).

Conclusion

Mercury-resistant bacteria were used to enzymatically reduce Hg (II) to water insoluble Hg (0). Seven mercury resistant strains of *Pseudomonas* were immobilized inside a 700 L packed bed bioreactor. A neutralized chloralkali electrolysis wastewater consisting of mercury concentrations of 3-10 mg/L was fed into the bioreactor (0.7 m^3/h up to 1.2 m^3/h). Within 10 hours of inoculation of the bioreactor, a mercury retention efficiency of 97% was obtained. Furthermore, the bioreactor outflow concentrations were below 50 µg Hg/L that fulfilled the discharge limit for industrial wastewater. The retention efficiency of the bioreactor was not affected by the inflow fluctuations, pH, or mercury concentrations (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000).

The mercury remediation process offers a promising way to extract mercury from polluted wastewater (Wagner-Dobler, Von Canstein, Li, Timmis, and Deckwer, 2000). This remediation method is applicable in ambient temperature and requires little electricity, which results in low operating costs. Moreover, this process, entitled "end-of-pipe technology," is effective on a

technical scale and applicable for mercury wastewater coming from chloralkali electrolysis factories, groundwater, soil wash water, and mining waters.

Case Study: Microbial Reduction and Oxidation of Mercury in Freshwater (Siciliano, O'Driscoll, and Lean, 2002).

Introduction

This experiment investigates the role of microbial mercury oxidation and reduction reactions in regulating dissolved gaseous mercury in Jacks Lake and Lake Ontario, two freshwater lakes (Siciliano, O'Driscoll, and Lean, 2002). No other researchers have examined the role of microbial activity in regulating freshwater patterns of dissolved gaseous mercury. This experiment studies the role of microbial activity on dissolved gaseous mercury. The experimenters hypothesized that microbial activity is the source of dissolved gaseous mercury and reduces levels of dissolved gaseous mercury (Siciliano, O'Driscoll, and Lean, 2002).

Materials and Methods

The dissolved gaseous mercury concentrations, microbial mercury reductase, and oxidase activity was measured between 45 and 90 minutes at two lakes: Jack's Lake, located approximately 200 km northeast of Toronto, Canada, and Lake Ontario (Siciliano, O'Driscoll, and Lean, 2002). Samples of water from Jack's Lake were collected from a fiber glass boat 15 cm below the surface by placing a narrow mouth Teflon bottle directly into the water. A Go-Flo sampler was used to measure Lake Ontario water (Siciliano, O'Driscoll, and Lean, 2002). The dissolved gaseous mercury was analyzed by a Tekran Zero Air Generator through a 1L water sample contained in a glass graduated cylinder (Siciliano, O'Driscoll, and Lean, 2002). The gas was analyzed for elemental mercury using a Tekran 2537A instrument. The Tekran 2537A is an instrument that first amalgamates mercury into a pure gold cartridge and then thermo desorbs the mercury so that a cold vapor atomic fluorescence spectrophotometry can analyze the gas every 5 minutes (Siciliano, O'Driscoll, and Lean, 2002).

Results and Conclusion

The microbial reduction and oxidation of elemental mercury presents an opportunity to reduce toxic levels of mercury in aquatic ecosystems. A microbial mercury reduction reaction involving the conversion of mercury (Hg^{2+}) to Hg (0) was observed. It was observed that the microbial mercury reductase activity increased when the dissolved gaseous mercury increased. The mercury oxidase activity that involves the conversion of Hg (0) to Hg (2+) increased as dissolved gaseous mercury concentrations decreased in the mid-afternoon. These results suggest that mercury oxidase activity was linked to hydrogen peroxide (H_20_2) diurnal patterns (Siciliano, O'Driscoll, and Lean, 2002).

After hydrogen peroxide was placed in Lake Ontario water, the mercury oxidase activity increased by 250%. By 60 minutes, dissolved gaseous mercury decreased to 28% of its initial concentration (Siciliano, O'Driscoll, and Lean, 2002). However, two hours after the addition of the hydrogen peroxide in the lake, the mercury oxidase activity declined, but the mercury reductase and dissolved gaseous mercury increased. Four hours after the addition of hydrogen peroxide, mercury reductase and dissolved gaseous mercury levels returned to their original levels (Siciliano, O'Driscoll, and Lean, 2002).

These results suggest that in the morning, microbial activity produces dissolved gaseous mercury and the photoreduction of Hg^{+2}. As hydrogen peroxide increases in concentration, a biological decrease in dissolved gaseous mercury occurred throughout the afternoon (Siciliano, O'Driscoll, and Lean, 2002). Results of this study helps in the understanding of the effects of microbial reduction and oxidation of mercury to dissolved gaseous mercury in lakes, which is beneficial to the study of mercury remediation.

Summary

Mercury is a metal that can exist in three forms, metallic (Hg)(0), mercurous (Hg^{2+}), and mercuric (Hg2+). Toxicity of mercury is due to the ability of both organomercurial compounds and inorganic forms of mercury and their high affinity to bind to membranes (Lovely, 2001). Organomercury compounds possess the ability to bind to membranes because they are lipid soluble. These characteristics allow mercury compounds to induce damage to membranes and inactivate periplasmic and cytoplasmic enzymes within a cell. Moreover, breathing mercury vapor has been documented to cause respiratory and acute renal failure. Organomercurial, in the form or methyl mercury (CH3Hg+), is also problematic for living organisms. This metal species is the most toxic form of mercury because it has a high affinity to damage the central nervous system, has high lipid solubility, and has the ability to reside in biological tissue for long periods of time. This compound can efficiently distribute throughout the body. Unlike methyl mercury, inorganic forms of mercury, such as HgCl, is less efficient at crossing biological membranes. Inorganic forms of mercury primarily travel to visceral tissues; therefore the lower portions of the body are generally affected by inorganic forms of mercury (Lovely, 2001). Mercuric forms affect the distribution rate and toxicity level of this metal within the body.

The methods used to reduce mercury compounds from sites were also discussed in this study. The reduction of Hg (II) to Hg (0) is a way to remove oxidized mercury compounds and to reduce soluble mercury (Hg) from the atmosphere (Lovely, 2001). As stated previously, mercury-resistant bacteria like *Pseudomonas aerginosa* can reduce Hg (II) to Hg (0). Reduction of mercury from Hg (II) to Hg (0) allows a decrease in the mercury concentration from a contaminated aquatic system. Experts suggest that this method may serve as an effective in situ remediation technique. Additionally, studies show that two-plasmid encoded membrane proteins can assist with the reduction of cellular permeability to Hg (II) through a bacteria strain *Enterobacteria aerogenes* (Lovely, 2001).

In the discussed case study, *"Removal of Mercury from Chemical Wastewater by Microorganisms in Technical Scale,"* the reduction of mercury Hg (II) to Hg (0) took place with the use of mercury-resistant bacteria with a cytoplasmic mercuric enzyme that was encoded by a mer A gene. DNA sequences consist of operons and genes. Operons are groups of genes that produce vital proteins for cells. There are two kinds of genes, structural and regulator genes, in an operon. Structural genes code for proteins and can form a single mRNA molecule during transcription. Regulator genes code for proteins can regulate other genes. Today, the full or partial DNA sequences of at least 10 mer operons are known (Lovely, 2001). The discovered DNA sequences are derived from different gram-negative bacteria species. However, they possess similar mer genes. Studies show operons are not found in eukaryotes. There are more recognized mer operons in gram-negative bacteria than in gram-positive bacteria. The presence of mer genes have been identified by gene-probing isolated bacteria species from mercury polluted and non-polluted environmental sample. Research also shows mer gene expression can occur in situ (Lovely, 2001). Moreover, studies are examining mer mRNA production and

mercury volatilization in environmental water samples. The mer mRNA is known to be affected by microbial activity and not affected by bacteria resistance. Mercury resistant bacteria and mer genes are important factors in the biocycling of mercury within the environment.

The case study entitled "*Microbial Reduction and Oxidation of Mercury in Fresh Water Lake*" examined the effects of microbial mercury oxidation and reduction processes to dissolved gaseous mercury in freshwater lakes (Steven D. Siciliano, Nelson J. O'Driscoll, and D.R.S, 2002). Studies show that decrease in dissolved gaseous mercury from lakes and rivers occurs because of the invasion of elemental mercury in the environment. Bacteria that are often located in freshwater lakes maintain an elemental level of mercury reductase activity that is able to reduce low concentrations of mercury from Hg^{2+} to Hg^{0}. It was discovered through the experiment that microbial activity produces dissolved gaseous mercury and is affected by the concentration of gaseous mercury.

These case studies highlight the methods used to effectively bioremediate mercury. Current studies show that increasing the number of plasmids containing mer genes and using permeabilized *E coli* cells that express mercuric reductase can facilitate the volatilization of mercury Hg (0), which helps with the removal of mercury from aquatic systems (Lovely, 2001). The re-engineering of the mer A. gene in the plant, *Arabidopsis thaliana*, created a mercury volatizing plant (Lovely, 2001). This method is useful from removing and immobilization of toxic metals in plant biomass.

Nevertheless, current approaches to bioremediating mercury rely upon microbial biosorption or ion-exchange resins. However, these strategies hold limitations because they lack sorption specificity in the presence of other metal ions and can be influenced by pH and the presence of metal ion-chelating agents. Biosorption of mercury (Hg^{2+}) is enhanced with the expression of mercuric ion transport proteins. The expression of mer T. and mer P. genes in *Escherichia coli* can enhance Hg (II) accumulation (Lovely, 2001). *Pseudomonas aeruginosa* can further assist with the biosorption of mercury (Lovely, 2001). The biosorption of mercury (Hg II) compounds allow immobilization of mercury ions to occur, which is beneficial to the environment (Lovely, 2001). In addition, immobilization of mercury is better than volatilization of mercury because studies show immobilization allows better revitalization of contaminated sites.

Implementation of bioremediation methods should be done with caution because many sites contain variables, such as multiple metals, organic compounds, and organisms, that affect the productivity of bioremediation approaches. Therefore remediation of contaminated sites usually requires a combination of many different approaches. Consequently bioremediation of mercury is complex and requires great understanding of microbial mercuric reactions within different environments.

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