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CHROMIUM (VI) BIOTREATMENT IN SOIL

by

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INTRODUCTION

Chromium is widely used in diverse industries and its inappropriate disposal practice has resulted in the release of this metal into the environment (1). Chromium has become one of the toxic metals most frequently detected in contaminated environments. The potential for adverse human health effects has led to increased public concerns over chromium contamination. Chromium exists in a variety of oxidation states, from 0 to +6. However, in natural environments only hexavalent chromium Cr(VI) and trivalent chromium Cr(III) are stable species. Cr(VI) is much more hazardous due to its carcinogenicity, mutagenicity and mobility, than the insoluble trivalent chromium compounds. Cr(III) is considered to be relatively innocuous and even essential to human health in minute quantities (2). Conventional chemical and electrochemical techniques for Cr(VI) removal are all based on reduction of Cr(VI) to Cr(III) and then precipitation of it as chromium hydroxide. The effective reduction of Cr(VI) normally requires an acidic reaction environment (pH<3), and the complete conversion is dependent on the concentration and type of reducing agents employed. However, applications of these techniques have limitations in terms of cost, effectiveness and sludge production. Recently the potential for the biotreatment of Cr(VI) wastes has received increased attention because the microbially mediated processes may offer a cost-effective alternative to chemical treatment. There are several biological mechanisms which may be suitable for metal treatments, including transformation, extracellular binding, complex formation, biosorption, and intracellular accumulation (3). Considering the more immobile and less toxic characteristics of Cr(III), the microbial reduction of Cr(VI) to Cr(III) appears to hold the most promise for the development of an innovative biotreatment technology. This reductive biotransformation not only leads to Cr(VI) detoxification but precipitates the metal in soils, therefore minimizing its potential risk to human health and impacted ecosystem through decreased toxicity and exposure.

Cr(VI) REMEDIATION BY MICROBIAL REDUCTION

Microbial reduction of Cr(VI) to Cr(III) is capable of occurring through two different mechanisms. One is enzymatic reduction in which microorganisms mediate electron transport to Cr(VI) either for anaerobic respiration or for detoxification. The other is an indirect reduction of Cr(VI) by reduced metabolic products such as H_2S. Both mechanisms have been explored for remediation of Cr(VI) contaminated environments, with more attention on the enzymatic process.

Cr(VI) as an electron acceptor

Microbial reduction of Cr(VI) is widely observed in both aerobic and anaerobic environments. The aerobic reduction of Cr(VI) is generally catalyzed by soluble enzymes associated with NADH as an electron donor or a co-enzyme (Table 1). Although the physiological function of microbially mediated electron transfer to Cr(VI) under the aerobic conditions has not been clearly understood, aerobic reduction of Cr(VI) is considered to be a cellular defense mechanism. This is because insoluble Cr(III), as an extracellular reduction product, is excluded from biological cells. Despite the lack of explicit explanations for its physiological role, the use of oxygen for microbial respiration suggests that aerobic reduction of Cr(VI) happens mainly as a side activity of microbial metabolism. However, it is believed that anaerobic reduction of Cr(VI) occurs when Cr(VI) acts as a terminal electron acceptor for microbial respiration (Table 1). From a thermodynamic point of view, Cr(VI) may serve as a competitive electron acceptor for anaerobic respiration, with the redox potential (+560 mV for CrO_4^{2-}/Cr^{3+}) only slightly less favorable than that of Fe(III) (+760 mV for Fe^{3+}/Fe^{2+}) and nitrate (+740 mV for NO_3^-/N_2), and far more favorable than that of sulfate (-230 mV for SO_4^{2-}/H_2S). Studies of the anaerobic microbial reduction of Cr(VI) indicates that Cr(VI) can serve as a terminal electron acceptor for reoxidation of respiratory chains during anaerobic metabolism. Further studies observed that membranes

associated cytochrome b, c, and d and cytochrome c_3 in soluble proteins are specifically involved in the transfer of electrons to Cr(VI) by microorganisms (4).

Although Cr(VI) reduction has been extensively demonstrated under anaerobic conditions, no cell growth has been observed to depend on Cr(VI) reduction (4). The results suggest that the energy yielded from Cr(VI) reduction may not be conserved in a manner which supports anaerobic cell growth. To maintain Cr(VI) reduction under anaerobic environments, the Cr(VI)-reducing microorganisms may thus require an alternative or intermediate electron acceptor. This is consistent with findings that anaerobic reduction of Cr(VI) commonly takes place in media containing fermentable organic compounds or complex media like nutrient broth, soy broth, and casamino acids. Thermodynamic calculations explain that oxidation of fermentable organic compounds with Cr(VI) as an electron acceptor is more energetically favorable than typical fermentation for the metabolism of glucose. As shown in Table 2, aerobic respiration is more energetically favorable than any of the fermentative oxidations. Under aerobic conditions, thus, Cr(VI) is reduced only via an abbreviated electron transport chain using NADH as an electron donor (5). Cr(VI) reduction is usually enhanced with a decrease in dissolved oxygen (DO) level, and the inhibitory effect of DO on microbial reduction of Cr(VI) has been quantitatively described (5). Under anaerobic environments, on the other hand, complete glucose oxidation with Cr(VI) as an electron acceptor, partial fermentation of glucose to acetate with Cr(VI) as an electron acceptor, and fermentation with Cr(VI) as a minor electron sink are all more energetically favorable than fermentation not involving Cr(VI) reduction.

Cr(VI) reduction by metabolic products

In addition to enzymatic reduction of Cr(VI), Cr(VI) can also be reduced by microbial metabolic products, including extracellular products and intracellular reducing agents. Cr(VI) is capable of entering bacterial cells via sulfate transport systems (6). Once Cr(VI) enters the cell it

may react with various cellular components such as sulfhydryl groups, and be reduced to Cr(III). It is believed that intracellular Cr(III) can not be eliminated from cells as long as the cell membrane remains intact. In terms of the characteristic of this redox reaction, the bacterial cell acts more like a chemical reducing reagent. Because the microbial metabolism would gradually terminate following continuous depletion of cellular reducing agents, the intracellular reduction of Cr(VI) appears to have a limited capacity . Thus, this mechanism has little applicability as an effective approach for biotreatment of Cr(VI).

However, recent progress suggests that extracellular reduction of Cr(VI) by reduced metabolic products can be developed as a useful biotreatment process. Cr(VI) reduction readily occurs in the presence of ferrous iron and sulfide regardless whether they are generated from abiotic or biotic sources. The reduced Cr(III) may precipitate in the form of chromium hydroxide under neutral to alkaline conditions. Although extracellular reducing compounds such as cysteine, glutathione, and other organic compounds are capable of reducing Cr(VI), most studies explored for bioremediation of Cr(VI) are focus on the use of H_2S produced from sulfate-reducing bacteria to remediate Cr(VI). Laboratory studies indicate that sulfate-reducing bacteria alone can create sufficient amounts of H_2S to ensure Cr(VI) reduction in natural environments (7). Since chromium sulfide is unstable, the reduced chromium is likely to deposit quickly as hydroxides. The concept of Cr(VI) reduction by sulfate-reducing bacteria has been developed to remediate Cr(VI)-contaminated wastewater (8). The microbial process was shown to tolerate Cr(VI) concentrations as high as 2,500 mg/L and reduced Cr(VI) to Cr(III) as amorphous precipitates which were attached to the bacterial surfaces. An indirect mechanism of Cr(VI) reduction, due to the generation of H_2S by the microbial consortia, was postulated. Although a successful application of this treatment process has been demonstrated in a pilot scale, the challenges remain. Since H_2S produced from bacterial respiration is re-oxidized by Cr(VI), the system redox potential that is required for active growth of sulfate-reducing bacteria cannot be achieved until the majority

of Cr(VI) has been removed. To maintain the microbial activity of Cr(VI) reduction in the system, therefore, interruption of Cr(VI) loads is repeatedly needed in practical operations. This disadvantage will limit its service for in situ biotreatment of Cr(VI).

Cr(VI) reduction coupling to carbon oxidation

Microorganisms are able to oxidize a variety of organic compounds for aerobic reduction of Cr(VI). The compounds serving as electron donors for Cr(VI) reduction include natural aliphatic compounds, mainly low-molecular-weight carbohydrates, amino acids and fatty acids, as well as alien compounds such as aromatic compounds (Table 3). Analogously, anaerobic reduction of Cr(VI) also demonstrates versatility with regard to electron donors. In addition to fermentable sugars, Cr(VI)-reducing anaerobes are also capable of using fermentation end products, such as formate, acetate, pyruvate, lactate, and ethanol as electron donors (Table 3). Benzoate, a common fermentation intermediate of aromatic compounds, has been observed to support reduction of Cr(VI) (12). The occurrence of such a wide variety of electron donors sustaining Cr(VI) reduction suggests that a common metabolic intermediate NADH or hydrogen, may serve as the direct electron donor for Cr(VI) reduction, while the other compounds may merely serve as precursors to produce these reducing agents via catabolic processes. Direct Cr(VI) reduction with hydrogen and NADH has been observed in both cell suspensions and cell-free fractions (4, 9).

In order to evaluate the feasibility of a bioprocess for treatment of Cr(VI), the mass relationship between electron donors and Cr(VI) should be understood. During the aerobic reduction of Cr(VI), the amount of electron donor oxidized should far exceed the amount theoretically required for Cr(VI) reduction due to the presence of molecular oxygen as a competitive electron sink. A good example of this is the reduction of Cr(VI) driven by phenol oxidation (10). Theoretically, oxidation of 1 mM phenol to carbon dioxide with complete flow of electrons to Cr(VI) should result in the reduction of 9.3 mM Cr(VI) according to following reaction:

$$3C_6H_6O + 28CrO_4^{2-} + 122H^+ = 28Cr^{3+} + 18HCO_3^- + 61H_2O \quad (1)$$

However, a statistical analysis of the experimental data revealed that consumption of 1 mM phenol resulted in actual reduction of 1.03 mM Cr(VI), a value much less than stoichiometrical requirement, suggesting that molecular oxygen is a major electron acceptor during the microbial oxidation of phenol. Therefore, to achieve aerobic biotreatment of Cr(VI), much more amounts of carbon sources are required than that of theoretical calculations.

Under anaerobic conditions, on the other hand, Cr(VI) may be the only electron acceptor, and thus the coupling Cr(VI) reduction to electron donor oxidation may proceed exactly according to the stoichiometry. When H_2 is used as the electron donor for Cr(VI) reduction, the molar ratio of H_2 consumption to Cr(VI) loss is 1.74±0.13 (11), a good agreement with the following formula:

$$3H_2 + 2CrO_4^{2-} + 10H^+ = 2Cr^{3+} + 8H_2O \quad (2)$$

The anaerobic biodegradation of benzoate with the transport of electrons to Cr(VI) illustrates another stoichiometrically balanced reaction between the electron donor and acceptor (12). Figure 1 shows the plot of the cumulative amount of benzoate degraded versus the Cr(VI) reduced. A statistical analysis indicates a strong linear relationship between the two parameters (R^2=0.98): 1.0 mM benzoate degraded = 10 mM Cr(VI) reduced. This result suggests that the benzoate oxidation during anaerobic reduction of Cr(VI) closely follows the stoichiometric formula:

$$C_7H_6O_2 + 10CrO_4^{2-} + 43H^+ = 10Cr^{3+} + 7HCO_3^- + 21H_2O \quad (3)$$

However, predicting or even evaluating the stoichiometric relationships under a mixed electron donor/acceptor in the environment may prove difficult. The rate and extent of electron donor oxidation and Cr(VI) reduction may depend on a variety of geochemical, ecological and physiological variables associated with the particular environmental setting.

Cr(VI) reducing microorganisms

Cr(VI) reduction has been observed with a large number of bacterial genera, most of which are facultative and ubiquitous in the environment. All of the listed Cr(VI) reducers in Table 3 are heterotrophic and are members of commonly occurring soil genera, such as *Pseudomonas*, *Bacillus*, and *Escherichia*. Usually a higher cell density of the cultures results in a greater rate of Cr(VI) reduction. However, cell growth is not necessarily required for Cr(VI) reduction to occur. Mass balance analysis indicates that Cr(VI) is ultimately and quantitatively reduced to Cr(III) although Cr(V) has been detected as a transient intermediate. Facultative microorganism such as *Agrobacterium, Bacillus, Escherichia,* and *Pseudomonas* can reduce Cr(VI) in the presence of oxygen and after the available oxygen is consumed. Others like *Enterobacter* can reduce Cr(VI) only under anaerobic conditions, and immediately lose the ability to reduce Cr(VI) in the presence of molecular oxygen despite good growth under aerobic conditions. The sulfate reducer *Desulfovibrio*, an obligatory anaerobe, is also capable of using Cr(VI) as a terminal electron acceptor (11). In addition, Cr(VI) is reduced by microbial consortia in soils, under both aerobic and anaerobic conditions. The mixed cultures reportedly reduce Cr(VI) to a greater extent than individual pure cultures. The dominance of facultative microorganisms in Cr(VI) reduction has a distinct advantage for biotreatment of soils in which the location of the oxic-anoxic interface may vary as a result of climate variations, human actions, and other biotic activities.

BIOPROCESS CONTROL PARAMETERS

In addition to the selection or cultivation of the appropriate bacterial strains, the bioprocess parameters that control microbial activities for Cr(VI) reduction must be evaluated and optimized for growth of the organisms, enzyme generation, and occurrence of Cr(VI) reduction. This process optimization will facilitate the systematic design and operation of the treatment unit.

Cr(VI) concentration

Cr(VI) may inhibit microbial metabolism, as demonstrated by decreases in cell growth, carbon utilization and Cr(VI) reduction rate (4). At high concentrations of Cr(VI), bacterial cultures show no significant cell growth, and a net decrease in active cells during the Cr(VI) reduction process. The simultaneous loss of the capabilities for glucose utilization and Cr(VI) reduction have been observed in a bacterial culture with high concentrations of Cr(VI). Studies using various pure cultures demonstrated that the time required for equivalent Cr(VI) reduction increased with the increase in the initial Cr(VI) concentration. However, some microorganisms have the ability to effectively reduce Cr(VI), even at a concentration as high as 500 mg/L. The adverse effects of high Cr(VI) concentrations on Cr(VI) reduction suggest that a suitable process design may be important to minimize the impact of high Cr(VI) concentrations.

Electron donors

The rate and extent of Cr(VI) reduction is dependent on the type and concentration of electron donors. In pure culture *Enterobacter* HO1, the use of complex electron donors such as casamino acids, tryptone, or yeast extract resulted in faster Cr(VI) reduction than single compound electron donors such as low-molecular weight acids, sugars or tricarboxylic acid cycle intermediates (13). Microorganisms may also utilize endogenous electron reserves for Cr(VI) reduction when external carbon sources become depleted. A benzoate initiated system showed that Cr(VI) reduction proceeded continuously in the absence of measurable external electron donor (benzoate), but at a significantly lower rate (12). In addition, native organic matter such as grass or cow manure have been tested for the ability to support biological Cr(VI) reduction in soils. In soils with a low organic matter content, Cr(VI) reduction was only increased slightly when the soil suspension was amended with 10% manure (14), suggesting that the absence of adapted microbial populations in the soil may limit the rate of Cr(VI) reduction. However, it has been reported that amendments with 2.2% cattle manure in an enriched system immediately increased the percentage

of Cr(VI) removal from 51% to 98% (15). A further comparison demonstrated that yeast extract, grass and other readily degradable organic matter supported even more rapid rates of microbial Cr(VI) reduction (16).

Competing electron acceptors

Observation of Cr(VI) reduction by facultative bacteria has shown that the presence of molecular oxygen can suppress Cr(VI) reduction, but does not completely terminate the transport of electrons to Cr(VI). A reduced level of dissolved oxygen may result in an increase in the rate of Cr(VI) reduction and the highest rate for Cr(VI) reduction is always obtained under anaerobic conditions with facultative microorganisms. An uncompetitive inhibition behavior for dissolved oxygen toward Cr(VI) reduction has been shown using the Lineweaver-Burk method, and the anaerobic reduction of Cr(VI) showed a maximum specific reduction rate approximately twice that observed under aerobic conditions (5). On the other hand, the aerobic bacteria usually do not have the capability for anaerobic reduction of Cr(VI) and the strict anaerobes will completely lose their activity in the presence of molecular oxygen.

Sulfate and nitrate have not been reported to inhibit aerobic reduction of Cr(VI) (4). Cr(VI) reduction was not inhibited with sulfate up to 96 mg/L and nitrate at 12 mg/L in the culture of *Pseudomonas*, while Cr(VI) reduction in *Bacillus* was not affected even with concentrations of sulfate and nitrate up to 1000 mg/L. However, under anaerobic conditions, a sulfate-reducing culture of *Desulfovibrio* was shown to reduce Cr(VI) in the presence of 5 g/L sulfate, but concentrations of 24 mg/L sulfate or 300 mg/L nitrate inhibited anaerobic reduction of Cr(VI) in *Enterobacter*. Cr(VI) reduction in anaerobic cultures of *Escherichia coli* was resistant to sulfate and nitrate up to levels of 8 g/L. In the studies with soil microcosms containing mixed native populations, Cr(VI) reduction was not affected by the presence of nitrate up to a concentration of 300 mg/L (12). Analysis of the microcosm system indicated that Cr(VI) and nitrate were concurrently used as the electron acceptors for benzoate oxidation without preference.

Thermodynamic calculations also illustrate the oxidation of benzoate with transport of electrons to Cr(VI) or nitrate yields very close standard free energies ($\Delta G^{0'}$=-499 and -510 kcal/mole benzoate, with Cr(VI) and nitrate as electron acceptors, respectively), supporting the concept of parallel transfer of electrons to Cr(VI) and nitrate in mixed or complex microbial system.

Other toxic metals and organic toxicants

High concentrations of heavy metals such as lead, mercury, copper, cadmium, and others are known to inhibit microbial metabolism. Cr(VI)-reducing microorganisms have been shown to be susceptible to certain heavy metal ions (4, 9). Cr(VI) reduction in *Enterobacter* was completely inhibited by 30 mg/L of Zn^{2+}, and 30 mg/L of Cu^{2+} decreased the reduction rate to 70% of the activity observed in the culture without added metals. An addition of 50 mg/L Zn^{2+} or 190 mg/L Cu^{2+} in the culture of *Escherichia coli* also reduced the reduction rate to approximate 80% of the activity observed in the absence of the metals, while Cd^{2+} or Pb^{2+} levels up to 20 mg/L showed no inhibition of Cr(VI) reduction. Cultures of *Desulfovibrio* reduced Cr(VI) at the same rate regardless of the presence of 11 metals (0.1 mM each) including nickelous chloride, cuprous chloride, zinc chloride, magnesium sulfate, vanadyl sulfate, sodium vanadate, sodium molybdate, and sodium selenate (11). Strong inhibition of Cr(VI) reduction in *Pseudomonas* by Hg^{2+} and Ag^{2+} was shown to be noncompetitive, with an inhibitory constant (K_I) equal to 20 μM for both metal ions. The same strain also reduced Cr(VI) without interference from the reduced product, Cr(III), at a concentration of 10 mg/L. In addition to metal ions, aromatic compounds were also observed as co-contaminants in Cr(VI) polluted streams and sites. Phenol and p-cresol at 5 mM and 2-chlorophenol at 2 mM severely inhibited Cr(VI) reduction and cell growth. Anaerobic cultures seem to be more susceptible to toxicity effects than the aerobic cultures. Toxicity studies using phenol, p-cresol and 2-chlorophenol indicated that the concentrations that caused 50%

decreases in rates of Cr(VI) reduction under aerobic conditions are nearly 1.5 times more toxic under anaerobic conditions.

Soil characteristics

Soils are composed of organic matter, inorganic matrix, soil atmosphere, water, plant roots and living microbial populations. The nature and the percentage of these contents may influence the performance of Cr(VI) biotreatment. In addition to the factors regulating abiotic Cr(VI) transport and fate processes in soils (such as sorption, anion exchange, and chemical transformation), parameters governing the rate and extent of Cr(VI) biotransformation in soils may include soil moisture, organic matter content, nutrient availability, redox potential, pH and salinity. To optimize microbial transformations of Cr(VI) in situ, these parameters must be adjusted to enhance development of large populations of Cr(VI)-reducing microorganisms and to bring these organisms into intimate contact with Cr(VI).

Soil moisture strongly influences microbial activity in the soil. Generally speaking, soil moisture at 70% to 80% of field capacity allows for rapid movement of air into the soil, and thus maintains optimal aerobic metabolism. When soils become excessively dry, microbial activity can be inhibited or terminated. In soils saturated with water, the available oxygen can be quickly consumed, which limits aerobic activities but may enhance the anaerobic transformations of Cr(VI). Microorganisms in soil require carbon sources for Cr(VI) reduction and may need other nutrients for cell growth. Soil organic matter is generally composed of 25% to 35% readily decomposed organic materials or compounds which have a short life in soils, while the other 65% to 75% is composed of humic materials which are generally resistant to microbial degradation. The widespread observation that Cr(VI) reduction is enhanced by organic amendments suggest that the biodegradable carbon sources are a limiting factor for microbial reduction of Cr(VI) in soils (16). Addition of easily degradable organic matter may result in the competition for this resource between Cr(VI)-reducing and other microorganisms, and may also cause shortages of other

nutrients due to the increased microbial biomass. Animal manures may supply both microbial carbon sources and certain other nutrients. The finding that the amendment of cow manure improved microbial reduction of Cr(VI) suggests that it may serve as an economical nutrient resource for the biotreatment of Cr(VI) in soils.

The redox potential of soils generally varies from -0.3V to +0.8V dependent on the rates of soil aeration and microbial respiration. Because Cr(VI) reduction occurs over a redox potential range from -0.24V to +0.25V (4), the redox potential of soils does not appear to be a critical limiting factor for occurrences of Cr(VI) reduction. However, a low redox potential environment in soils following depletion of oxygen will definitely promote anaerobic reduction of Cr(VI). A pH range of 6 to 8 has been shown to support microbial reduction of Cr(VI) (4). Soil pH may be lowered by addition of ferrous or aluminum sulfate, whereas it can be raised by addition of agriculture limes. Salinity also affects microbial activities. High salinity soils with an electrical conductivity (EC) value greater than 8 dSm^{-1} will restrict activities of many microorganisms, while soils with a value of 2 dSm^{-1} or less may not be a problem to most microbial metabolism. The effect of salinity on microbial reduction of Cr(VI) has not been established but may also follow this general rule.

APPLICATION OF Cr(VI) BIOTREATMENT

The remediation of Cr(VI) contaminated soils is a complex and challenging task. In situ microbial cleanup of contaminants has been successfully utilized for more than 30 years to restore polluted sites including soils and groundwater. Currently, most applications of biotreatment have focused on oxidation transformations of organic wastes. The application of reductive biotransformation for bioremediation is less well developed and, to date, has been used most successfully for the biotreatment of chlorinated organic compounds and for nitrate removal. The extensive occurrence of Cr(VI) reducing microorganisms in both contaminated and uncontaminated

soils and sediments is evidence for the potential of in situ biotreatment processes. The occurrence of microbial reduction of Cr(VI) in a neutral pH range may offer the best promise for in situ bioremediation of Cr(VI). In situ treatment would concentrate chromium on the soils as Cr(III), which has greatly reduced environmental mobility and biological availability. Laboratory and field research is currently underway to develop effective reductive biotreatment techniques for Cr(VI) contaminated soil and water.

The biotreatment of Cr(VI) in soils may be enhanced through two approaches. One is stimulating native microorganisms for Cr(VI) reduction by adjusting environmental conditions, and the other is altering the microbial population by inoculating seed organisms. Both approaches have been employed to accelerate bioremediation of petroleum contaminated sites. Laboratory and field evidence also demonstrates the capability of these approaches for enhancing microbial treatment of Cr(VI).

Enhancement of indigenous microorganisms

The site environment can be adjusted to activate or enhance microbial reduction of Cr(VI) by supplying carbon sources, essential nutrients, and possibly other electron acceptors for stimulating bacterial growth. In soils with abundant organic materials, native microorganisms may use those easily degradable organic compounds as electron donors to reduce Cr(VI). As shown in Figure 2, significant reduction of Cr(VI) was observed in the absence of external organic compounds (Sewell and Shen, unpublished data). However, the addition of an appropriate electron donor further enhanced Cr(VI) reduction in the microcosms (Figure 2). Based on the observed curves of Cr(VI) reduction, sucrose was the most effective electron donor for bacterial transformation of Cr(VI), followed by lactate and acetate, and then the native organic compounds in soil. On the other hand, in oligotrophic soils, the addition of external electron donors may play an essential role in stimulating Cr(VI) reduction. Soils with both native microorganisms and inoculant showed insignificant Cr(VI) reduction in the absence of added electron donors (14).

When amended with carbon sources, however, the soil microorganisms rapidly reduced Cr(VI) to Cr(III). The rate of Cr(VI) reduction was also dependent on the nature of carbon sources. The addition of the easily decomposable yeast extract yielded a higher rate of Cr(VI) reduction, while a much lower rate of Cr(VI) reduction was obtained when grass and cow manure were used as carbon sources. Field test results also confirmed the requirement of carbon sources for effective biotreatment of Cr(VI) (15). The amount of Cr(VI) reduced was observed to be proportional to the cow manure loading and Cr(VI) removal percentages as high as 98% have been reported. The cow manure serving as the electron donor appeared to be a limiting factor for microbial reduction of Cr(VI) because its loading decided the extent of Cr(VI) reduction in the biotreatment system.

When native microorganisms are incapable of coupling oxidation of the supplied carbon sources to Cr(VI) reduction, the addition of an alternative electron acceptor may provide assistance (12). Microcosm tests observed that native microorganisms in oligotrophic soils were unable to reduce Cr(VI) when benzoate was provided as the carbon source. However, it has been noted that nitrate or oxygen may act as an initial stimulator for linkage of benzoate oxidation and Cr(VI) reduction. After depletion of nitrate or dissolved oxygen, the microorganisms still retained the capacity for benzoate degradation linked to Cr(VI) reduction. Since denitrifying organisms in the microcosms alone did not have the capability to reduce Cr(VI), Cr(VI) reduction in this system is probably attributed to microbial consortia, possibly including both denitrifers and Cr(VI) reducers. According to these findings, the oxic-anoxic conditions in soils may easily facilitate microbial reduction of Cr(VI) allowing a wide spectrum of carbon sources to serve as electron donors.

Other nutrients are reported to have less effect on Cr(VI) reduction in soils. This is not surprising since Cr(VI) reduction occurs without necessarily being coupled to the growth of microbial cells, and the microbial cells retain the normal ability to reduce Cr(VI) even without additions of nitrogen and phosphorous (4). Because Cr(VI) toxicity may lead to cell inactivation and loss of Cr(VI) reduction capacity, the stimulation of microbial growth to generate fresh cells in soils may be required under operational conditions. In addition to carbon sources, alternative

electron acceptors may also be critical to applications requiring cell growth, since significant growth of cells has not been observed during Cr(VI) reduction. Oxygen and nitrate appear to be the most appropriate electron acceptors for promoting microbial growth in Cr(VI) biotreatment applications. Oxygen could be supplied by sparging air in a batch mode or as a continuous feed. The batch mode addition is more economical and may also give better Cr(VI) reduction performance. This is because the feeding patterns result in cyclic changes in soil redox that may optimize aerobic cell growth and anaerobic Cr(VI) reduction. Nitrate is much more soluble than oxygen, hence it may be more economical to sustain microbial growth under denitrifying rather than aerobic conditions. This is especially true for biotreatment of Cr(VI) in deep soils because of the difficulties in delivering a significant mass of soluble oxygen to the contaminated zone, and the potential for degassing, and reactions with inorganic reducing compounds.

Inoculation of acclimated microorganisms

Another approach for enhancing Cr(VI) biotreatment is the addition of adapted microbes into Cr(VI) contaminated soils. There is little information describing the use of inoculation to promote microbial reduction of Cr(VI). One successful example reported the introduction of an adapted enrichment into microcosms that contained Cr(VI) and benzoate as the sole electron donor and acceptor (12). In the inoculated microcosms, Cr(VI) was rapidly reduced with concurrent degradation of benzoate following a lag period of 4 days for both Cr(VI) and benzoate. This biological activity was extended to more than 50 days following repeated addition of Cr(VI) and benzoate, demonstrating that the inoculant was capable of survival under the new environmental conditions. Although the inoculated microcosms continued to consume Cr(VI) and benzoate, the analogous microcosms which contained no inoculation showed no decreases in the amount of Cr(VI) and benzoate throughout the same period. In another case, a pure culture of *Pseudomonas*, a common strain known to be capable of Cr(VI) reduction, was inoculated into soil microcosms amended with Cr(VI) (16). The inoculant, however, failed to provide any better reduction of

Cr(VI) than was achieved by simply adding organic matter. Apparently, the addition of organic matter allowed native Cr(VI)-reducing microorganisms to accelerate the activity of Cr(VI) reduction, probably outcompeting the introduced organisms. Hence, microbial inoculation may be necessary only if the native Cr(VI)-reducing organisms are missing or are inactivated by high levels of Cr(VI). Many factors have been recognized to restrict the applicability of inoculation in bioremediation (17). In the case of Cr(VI) biotreatment, these limitations may include the delivery of the inoculum to the contaminated zone, adverse microbial interactions such as competition and predation from native organisms, and antibacterial substances in soils.

Advantages and disadvantages of in situ biotreatment

When biotreatment of Cr(VI) is carried out in situ, costs may be substantially reduced by eliminating the large energy inputs for excavation and shipment of contaminated soils. Conceptually, in situ treatment also reduces the risks associated with soil movement to workers and local residents. Where soil organic compounds are rich, Cr(VI) reduction may occur by soil microorganisms as natural attenuation. If electron donors become a limiting factor for microbial Cr(VI) reduction in soils, natural organic matter from economical sources can be added to improve microbial activity. Under certain conditions, microbial consortia can even utilize soil co-contaminants such as aromatic compounds as electron donors for Cr(VI) reduction, accomplishing simultaneous cleanup of metal and organic contaminants. Since Cr(VI) reduction by indigenous microbes produces no hazardous metabolites and occurs at a neutral range of pH, this biotreatment process should result in minimal impacts on the soil ecosystem.

Despite the potential advantages, in situ biotreatment of Cr(VI) requires detailed site examinations for geochemical, hydraulic, and microbial characterization. High levels of Cr(VI) may repress or inhibit microbial activities including Cr(VI) reduction. The rate and extent of microbial Cr(VI) reduction are greatly affected by temperature, soil moisture, co-contaminant and other conditions. In addition, extended growth of microbes can also plug the soil and thus reduce nutrient circulation. However, it should be emphasized that the biotreatment process may not

necessarily replace other treatment technologies, but may be combined with them or be used as a supplemental polishing process. The microbial process, together with widely reported abiotic reduction processes, may offer an efficient and cost-effective approach for in situ remediation of Cr(VI) contaminated soils.

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Figure 1. Cumulative Cr(VI) reduced versus cumulative benzoate degraded in an active enrichment of soil microorganisms with Cr(VI) and benzoate as the sole electron acceptor and donor. The results were reported by Shen et al (12).

Figure 2. Microbial reduction of Cr(VI) in microcosms constructed with soils from Norman Landfill, Norman, Oklahoma.(unpublished data)

Table 1. Properties of reductase involved in Cr(VI) reduction

Property	Aerobic reduction	Anaerobic reduction
Location		
Soluble	+	+
Membrane	-	+
Co-factor		
NADH	+/-	+
Cytochrome	-	+
Phosphorylation occurrence	+	+/-
Inhibition		
Oxygen	-	+
Nitrate	-	+/-
Sulfate	-	+/-
Electron donors		
NADH	+	+
Hydrogen	?	+
Sugars	+	+
Fatty acids	+	+
Endogenous reserves	+	+

+ Positive result

- Negative result

Table 2. Energy potentially available from various pathways for glucose metabolism (5)

No	Reactant	Product	$\triangle G^{o'}$ (KJ/electron transferred)
1	$C_6H_{12}O_6 + 6O_2$	$6CO_2 + 6H_2O$	-121
2	$C_6H_{12}O_6 + 8CrO_4^{2-} + 34H^+$	$8Cr^{3+} + 6HCO_3^- + 20H_2O$	-83
3	$C_6H_{12}O_6 + H_2O$	$CH_3COO^- + CH_3CH_2COO^- + HCO_3^- + H_2 + 3H^+$	-71
4	$C_6H_{12}O_6 + 2.7H_2O$	$0.67CH_3COO^- + 0.67CH_3CH_2CH_2COO^- + 2HCO_3^- + 2.7H_2 + 3.3H^+$	-60
5	$C_6H_{12}O_6 + 2H_2O$	$2CH_3CH_2OH + 2HCO_3^- + 2H^+$	-57
6	$C_6H_{12}O_6$	$OOCCH_2CH_2COO^{2-} + CH_3COO^- + H_2 + 3H^+$	-66
7	$C_6H_{12}O_6$	$2CH_3CHOHCOO^- + 2H^+$	-50
8	$C_6H_{12}O_6 + 2.7CrO_4^{2-} + 9.3H^+$	$2.7Cr^{3+} + 2CH_3COO^- + 2HCO_3^- + 6.7H_2O$	-109
9	$C_6H_{12}O_6 + 0.67CrO_4^{2-} + 0.3H^+$	$0.67Cr^{3+} + CH_3COO^- + CH_3CH_2COO^- + HCO_3^- + 1.67H_2O$	-96

Table 3. Genera of microorganisms capable of reducing Cr(VI)*

Genera	Substrate/redox condition/source
Achromobacter	acetate, glucose/anaerobic/undefined
Aeromonas	galactose, fructose, mannose, melibiose, sucrose, lactose, cellobiose, arabinose, mannitoldulcitol, sorbitol, glycerol/anaerobic/sewage
Agrobacterium	glucose, fructose, maltose, lactose, mammitol, glycerol/aerobic, anaerobic/soil
Bacillus	acetate, glucose/aerobic, anaerobic/soil
Desulfovibrio	H_2/anaerobic /undefined
Enterobacter	acetate, glycerol, glucose, casamino acid, malate, oxalate, pyruvate/anaerobic/sewage
Escherichia	acetate, glucose/aerobic, anaerobic/sewage
Micrococcus	acetate, glucose/aerobic, anaerobic/undefined
Pseudomonas	peptone, glucose, ribose, fructose, glycerol, fumarate, lactate, acetate, succinate, butyrate, ethylene,/aerobic, anaerobic/sewage, sediment, soil
Escherichia and *Pseudomonas*	phenol, chlorophenol, cresol, dimethylphenols, benzene, toluene/aerobic/soil, sewage
Undefined soil organisms	lactate, acetate, sucrose, ethanol, benzoate, grass, cow manure/anaerobic/soil

* Based on references (4, 11- 16)

Figure 1. Cumulative Cr(VI) reduced versus cumulative benzoate degraded in an active enrichment of soil microorganisms with Cr(VI) and benzoate as the sole electron acceptor and donor. The results were reported by Shen et al (12).

Figure 2. Microbial reduction of Cr(VI) in microcosms constructed with soils from Norman Landfill, Norman, Oklahoma.

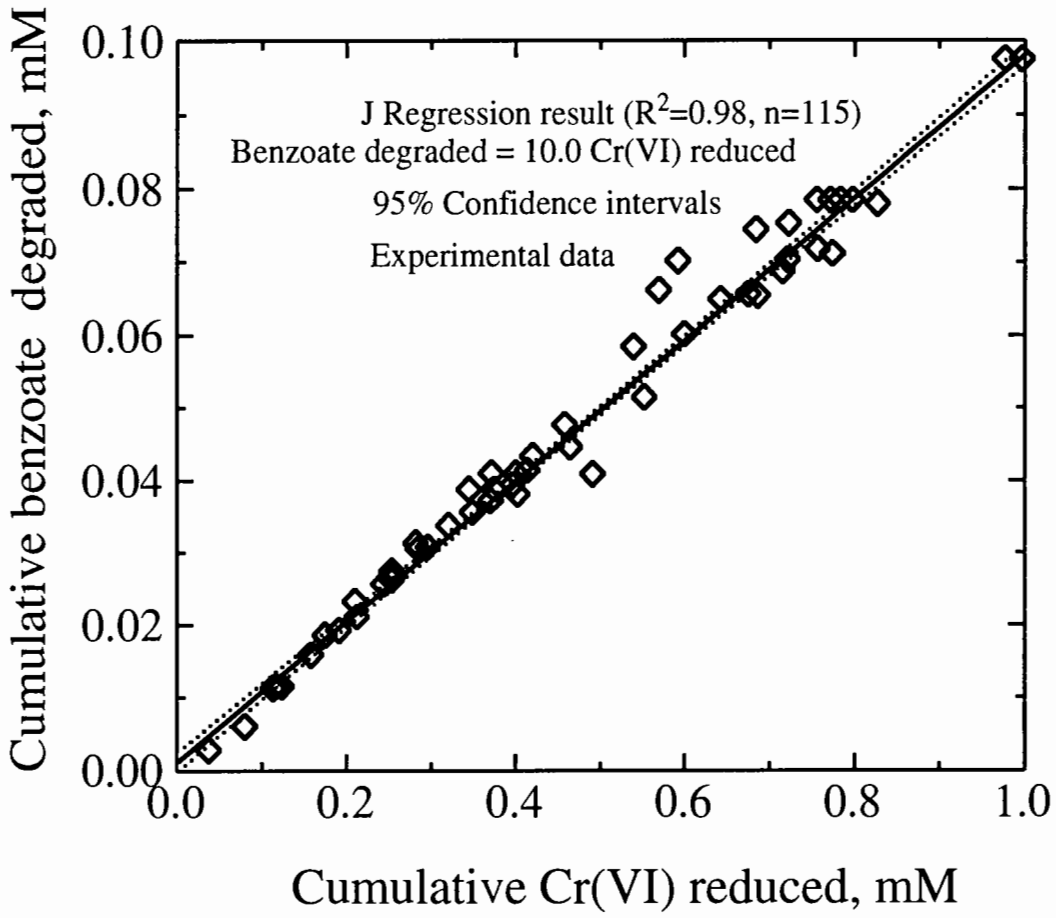
J Regression result (R^2=0.98, n=115)
Benzoate degraded = 10.0 Cr(VI) reduced
95% Confidence intervals
Experimental data
Cumulative benzoate degraded, mM
Cumulative Cr(VI) reduced, mM
0.00
0.02
0.04
0.06
0.08
0.10
0.0
0.2
0.4
0.6
0.8
1.0

Fig. 1

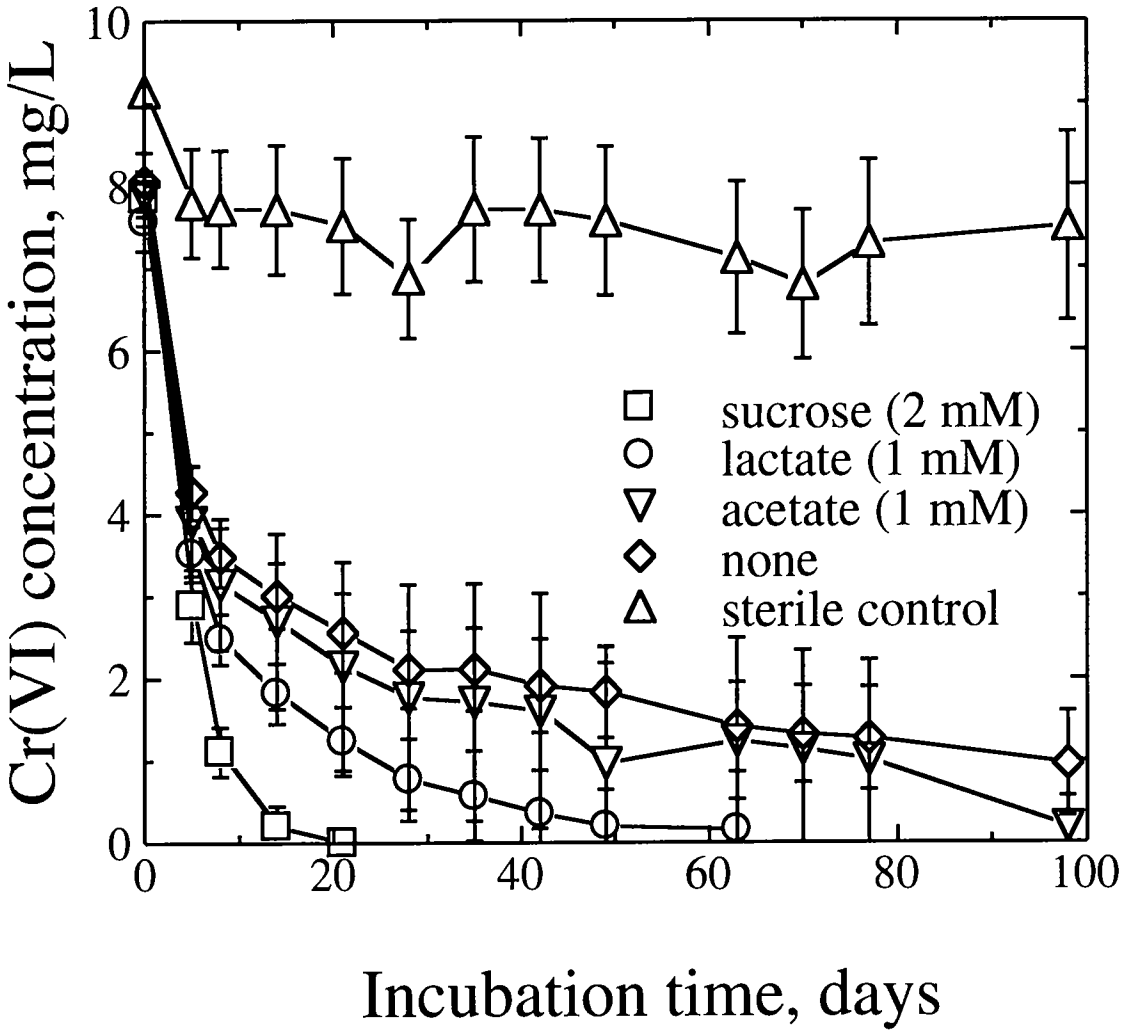
Cr(VI) concentration, mg/L
10
8
6
4
2
0
0
20
40
60
80
100
Incubation time, days
sucrose (2 mM)
lactate (1 mM)
acetate (1 mM)
none
sterile control

Fig. 2

TECHNICAL REPORT DATA

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16. ABSTRACT

Chromium is widely used in diverse industries and its inappropriate disposal practice has resulted in the release of this metal into the environment. Chromium has become one of the toxic metals most frequently detected in contaminated environments. The potential for adverse human health effects has led to increased public concerns over chromium contamination. Chromium exists in a variety of oxidation states, from 0 to +6. However, in natural environments only hexavalent chromium Cr(VI) and trivalent chromium CR(III) are stable species. Cr(VI) is much more hazardous due to its carcinogenicity, mutagenicity and mobility, than the insoluble trivalent chromium compounds. Cr(III) is considered to be relatively innocuous and even essential to human health in minute quantities (2). Conventional chemical and electrochemical techniques for Cr(VI) removal are all based on reduction of Cr(VI) to Cr(III) and then precipitation of it as chromium hydroxide. The effective reduction of Cr(VI) normally requires an acidic reaction environment (pH<3), and the complete conversion is dependent on the concentration and type of reducing agents employed. However, applications of these techniques have limitations in terms of cost, effectiveness and sludge production. Recently the potential for the biotreatment of Cr(VI) wastes has received increased attention because the microbially mediated processes may offer a cost-effective alternative to chemical treatment. There are several biological mechanisms which may be suitable for metal treatments, including transformation, extracellular binding, complex formation, biosorption, and intracellular accumulation (3). Considering the more immobile and less toxic characteristics of Cr(III), the microbial reduction of Cr(VI) to Cr(III) appears to hold the most promise for the development of an innovative biotreatment technology. This reductive biotransformation not only leads to Cr(VI) detoxification but precipitates the metal in soils, therefore minimizing its potential risk to human health and impacted ecosystem through decreased toxicity and exposure.

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