TABLE 2

Concentrations of metals in soils at which negative effects on N_2-fixation in cyanobacteria (blue-green algae) were observed

Experimental site		mg kg^{-1} soil					
		Zn	Cd	Cu	Ni	Pb	Cr
Woburn, UK[a]		114	2.9	33	17	40	80
Ultuna, Sweden[b]		230	0.7	125	35	40	85
Braunschweig 1, Germany[c]	L sludge	42–93	0.36–0.81	–	1.75–4.5	–	–
	H sludge	132–305	0.58–2.38	–	5.4–17	–	–
Braunschweig 2, Germany[d]	L sludge	26–81	0.4–0.88	–	1.5–4.6	–	–
	H sludge	86–240	0.7–2.15	–	3.7–16	–	–

[a] 50% reduction in N_2-fixation.
[b] >50% reduction in N_2-fixation.
[c] Braunschweig 1: 30% and 70% reduction in N_2-fixation in the Low (L) and High (H) rate of sludge application respectively.
[d] Braunschweig 2: 25% and 100% reduction in N_2-fixation in the L and H respectively.

unamended sludge also decreased the abundance of cyanobacteria, compared with NPK only. The large amount of available nitrogen and carbon may suppress N_2-fixation in cyanobacteria and may also favour competition by heterotrophic organisms [21].

Symbiotic nitrogen fixation

Traditionally, the most important agricultural associations involving *Rhizobium* or *Bradyrhizobium* include those with lucerne or alfalfa, pulses such as peas and beans and clover in grass–clover leys. For example, N_2-fixation by white clover (*Trifolium repens* L.) can amount to more than 200 kg N ha^{-1} yr^{-1} [2]. This is therefore an important source of nitrogen in low cost agroecosystems such as grass–clover leys where very little or no additional inorganic nitrogen fertilizers are required.

Effects of metals on clover growth and nitrogen fixation

Significant decreases in white clover yields in a mixed grass/clover sward were reported by Vaidyanathan [51] in plots of the experiment at Lee Valley contaminated predominantly by Zn seven years previously. Copper also decreased the yield of clover, but to a lesser extent, whereas Ni and Cr had no effect. When grown in monoculture at Woburn, white clover yields decreased by 60% on metal-contaminated sludge-treated plots compared to FYM-treated plots more than 20 years after the sludge had been applied [43] (Table 3).

Red clover failed almost completely at the three harvests taken in 1985 from some of the most metal-contaminated plots at Luddington, particularly those with 455 and 511 mg Zn kg^{-1} soil [30]. In the 'low' Zn treatments, with 238 mg Zn kg^{-1} soil, there was no decrease in yield. Other treatments with 118 and 91 mg Ni kg^{-1} soil also gave poor yields, but only at the first harvest. However, these plots also contained 128 and 104 mg Zn kg^{-1} soil, respectively, and it is likely that this combination of Zn and Ni caused the initial poor establishment. Similarly, at Gleadthorpe, yields of white clover decreased in Zn- and Cu-contaminated soils (Table 3). Zinc and Cu together at concentrations of (Zn:Cu mg kg^{-1}) 172:47, 173:107 and 209:94 decreased the clover yields by 20, 31 and 67%, respectively, compared to the controls [48]. Nickel by itself had no effect on yields at the largest concentration of 31 mg kg^{-1} soil, nor were there any yield decreases in any of the combined Zn and Ni treatments. This may be because, in all cases, the Zn concentrations were smaller than the concentrations at which yield reductions occurred in other treatments.

From the above studies, it is not possible to determine whether the metal effects reported on clover yield were due to phytotoxicity or due to an effect on N_2-fixation or on the rhizobia in these soils. No data were collected at the time on the effects of these metals on N_2-fixation and/or on the rhizobial population at Lee Valley, Luddington or Gleadthorpe. However, McGrath et al. [42] showed that the yield decreases at Woburn on the metal-contaminated soil were not due to phytotoxicity, as the clover yields could be restored to those in the control FYM-treated soil by the addition of nitrogen fertilizer. The reductions in clover yields were due to an effect on N_2-fixation which was reduced in a pot experiment by more than 50% in soils containing more than (mg kg^{-1}): 334 Zn, 99 Cu, 27 Ni and 10 Cd. It seems likely that the metal effects on clover yields at Lee Valley, Luddington and Gleadthorpe were also due to an effect on N_2-fixation rather than phytotoxicity, since the metal concentrations were similar to those found in the metal-contaminated soil at Woburn.

Rhizobium

Rhizobia are well adapted to life as free-living soil bacteria and can survive for a long time in the absence of the host [47]. Rhizobia have the ability to nodulate leguminous plants, but some nodules can be ineffective and never fix nitrogen due to a deficiency in either the host or symbiont. These ineffective nodules are usually small (<2 mm) and white in appearance because they lack leghaemoglobin. Effective nodules are large (>3 mm) and always pink in colour due to the large amount of leghaemoglobin present.

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Bioremediation of organic and metal contaminants with dissimilatory metal reduction

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SUMMARY

Dissimilatory metal reduction has the potential to be a helpful mechanism for both intrinsic and engineered bioremediation of contaminated environments. Dissimilatory Fe(III) reduction is an important intrinsic process for removing organic contaminants from aquifers contaminated with petroleum or landfill leachate. Stimulation of microbial Fe(III) reduction can enhance the degradation of organic contaminants in ground water. Dissimilatory reduction of uranium, selenium, chromium, technetium, and possibly other metals, can convert soluble metal species to insoluble forms that can readily be removed from contaminated waters or waste streams. Reduction of mercury can volatilize mercury from waters and soils. Despite its potential, there has as yet been limited applied research into the use of dissimilatory metal reduction as a bioremediation tool.

INTRODUCTION

The possibility of using bioremediation to restore environments contaminated with organics is well known [21]. Bioremediation may either be 'intrinsic bioremediation' which naturally takes place in contaminated environments or engineered [29]. For non-chlorinated organics, the common engineering practice is to enhance aerobic degradation of the contaminants by ensuring that the supply of O_2 and inorganic nutrients does not limit the rate of contaminant degradation [100]. Nitrate has been considered as an alternative electron acceptor for engineered bioremediation of ground water but has not found widespread use. This is because most engineered bioremediation of non-chlorinated organics deals with petroleum contamination and benzene, the most important petroleum-related ground water contaminant, does not appear to be readily degraded with nitrate as the electron acceptor [6,11,35,48,49,55]. Recent studies have demonstrated that Fe(III) may be an important electron acceptor in the intrinsic bioremediation of ground waters contaminated with non-chlorinated organics [7,67,74]. Furthermore, techniques for stimulating the activity of Fe(III)-reducing microorganisms to stimulate contaminant degradation have been developed [72].

In contrast to organics, metal contaminants are not 'biodegradable'. However, a number of important contaminant metals and metalloids are either less soluble or more volatile in the reduced state than they are in the oxidized state (Table 1). Thus, dissimilatory metal reduction may be a useful technique to precipitate or volatilize metal contaminants from polluted waters or waste streams. The purpose of this review is to summarize recent studies which have investigated the potential for using the metabolism of dissimilatory metal-reducing microorganisms as a tool for bioremediation of both organic and inorganic wastes.

TABLE 1

Reactions examplifying how dissimilatory metal reduction can be involved in bioremediation of contaminants

Reaction number	Reactants	Products
	Oxidation of organic contaminants coupled to Fe(III) reduction	
1.	Toluene+36 (Fe(III)+21 H_2O	$7HCO_3^-$+36 Fe(II)+43 H^+
	Reduction of soluble metal to an insoluble form	
2.	U(VI)+H_2[a]	U(IV)+2 H^+
3.	Cr(VI)+3/2 H_2	Cr(III)+3 H^+
4.	Se(VI)+[3 H_2][b]	Se(0)+6 H^+
5.	Pb(II)+[H_2]	Pb(0)+2 H^+
6.	Tc(VII)+[3/2 H_2]	Tc(IV)+3 H^+
	Reduction of soluble metal to a volatile form	
7.	Hg(II)+[H_2]	Hg(0)+2 H^+

[a]H_2 designates that molecular H_2 has been demonstrated to be an electron donor for reduction of the metal. However, microorganisms can also use organic electron donors for reduction of these metals.
[b][H_2] designates two electrons are donated from various organic electron donors; molecular H_2 has not been shown to be an electron donor for reduction of these metals in pure culture.

Intrinsic bioremediation of organic contaminants by Fe(III)-reducing microorganisms

TABLE 3

Minimum concentrations of metals in soils at which yields of clover or the population of *Rhizobium leguminosarum* biovar *trifolii* were decreased

Experimental site	mg kg^{-1} soil					
	Zn	Cd	Cu	Ni	Pb	Cr
Woburn, UK[a]	180	6.0	70	22	100	105
Gleadthorpe, UK[a]	281	–	150	–	–	–
Braunschweig 1, Germany[b]	200	1.0	48	15	–	–
Braunschweig 2, Germany[b]	130	0.8	27	11	–	–

[a] Clover yields.
[b] Rhizobial population.

Effects of metals on Rhizobium leguminosarum *biovar* trifolii

The decrease in clover yield reported by McGrath et al. [43] in metal-contaminated soil at Woburn was due to a lack of N_2-fixation as a result of ineffective nodules on clover plants [42], whereas plants grown in FYM-treated control soil had effective nodules. *Rhizobium leguminosarum* biovar *trifolii* isolated from nodules on clover plants grown in the metal-contaminated soil were found to be ineffective in N_2-fixation in plant infection tests in the absence of metals [25]. No effective rhizobia were present in the metal-contaminated plots, whereas the FYM-treated control plots, some 2 metres away, had effective rhizobia. In a further experiment where increasing numbers of effective *R. leguminosarum* bv. *trifolii* were added to metal-contaminated soil from Woburn, and the soils incubated for 2 months in a moist condition in the laboratory, no effective nodulation was obtained on clover plants where 10^7 cells g^{-1} soil or less were added [25]. Only with the additions of extremely large numbers of effective rhizobia (>10^{10} cells g^{-1} soil) did sufficient survive to give effective nodules on clover plants after 2 months. These workers concluded that effective clover rhizobia were unable to survive in the free-living state outside the protected root nodule environment in the metal-contaminated soil at Woburn. They also suggested that Cd, Zn and Cu were likely to be the most toxic metals to rhizobia.

Smith et al. [49] enumerated the population of indigenous *R. leguminosarum* bv. *trifolii* in soils from plots of the Luddington experiment. Even though all the plots had clover growing in them when the soil samples were taken, the numbers of rhizobia were an order of magnitude smaller in the high Zn-treatment compared to both the control soils. Rhizobial numbers also decreased in the high Cu soil, but to a lesser extent, whereas Cr and Ni had no effect (Fig. 1). Smith et al. [49] concluded from their results at Luddington that the order of decreasing toxicity to the rhizobial population was Zn > Cu > Ni > Cr. The metal concentrations reported by these authors in this soil were (mg kg^{-1}): 542 Zn, 34 Cu, 48 Ni and 160 Cr. No data were given for Cd. However, there is some dispute as to the metal concentrations in the high Zn plots since Chander and Brookes [10], using soils from the same plots for soil microbial biomass work, reported metal concentrations of (mg kg^{-1}): 281 Zn, 30 Cu, 41 Ni and 1.5 Cd. The Zn concentration was approximately half that reported by Smith et al. [49].

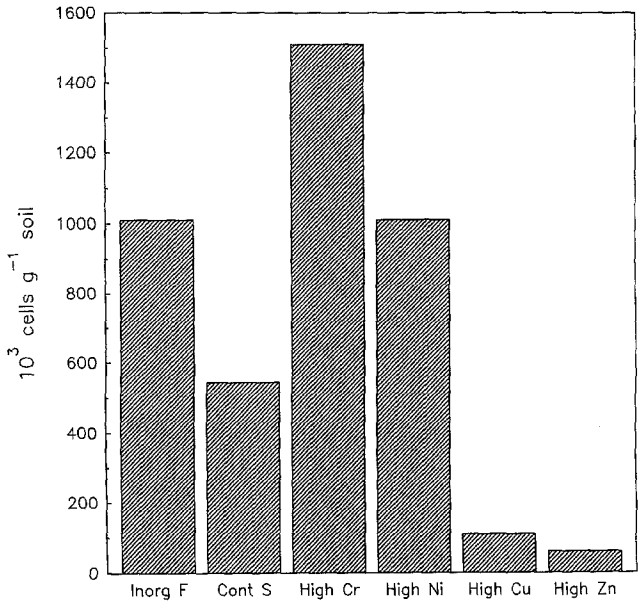

Fig. 1. Numbers of indigenous *Rhizobium leguminosarum* biovar *trifolii* in soils from Luddington (after Smith et al. [49]). Treatments: Inorg F = Inorganic fertilizers; Cont S = Control sludge; High = soils high in metals stated.

In the field experiment at Ultuna, *R. leguminosarum* bv. *trifollii* numbers were found to be an order of magnitude smaller in soil previously treated with metal-contaminated sludge (e.g. 2.5×10^3 cells g^{-1} soil) compared to the unfertilized soil (e.g. 1.9×10^4 cells g^{-1} soil) and FYM-treated soil (e.g. 1.8×10^4 cells g^{-1} soil) [38]. No legumes had been grown on this site for 30 years. Rhizobia isolated from clover plants in the metal-contaminated soil showed a distinct delay in nodulation in plant infection tests compared to isolates from plants grown in both the unfertilized and FYM-treated soils. Both the unfertilized and FYM-treated plots contained background metal concentrations, whereas those in the sludge-treated plot are the same as those given in Table 2. The large

development of anoxic conditions. Prior to the development of anoxic conditions, Fe(III) is generally the most abundant potential electron acceptor for organic matter oxidation in most soils and sediments [60]. Studies with pure cultures and highly purified enrichments have demonstrated that a wide variety of monoaromatic compounds can be oxidized to carbon dioxide with Fe(III) serving as the sole electron acceptor [58,62,67] and geochemical evidence has suggested that this type of metabolism (reaction 1, Table 1) is an important mechanism for the intrinsic bioremediation of organic contaminants [7,34,67,74,101].

For example, in the anoxic zone of a petroleum-contaminated aquifer there was a loss of aromatic hydrocarbons which was accompanied by an accumulation of isotopically-light carbon dioxide [67]. This suggested that the isotopically-light aromatic hydrocarbons were being oxidized to carbon dioxide. The accumulation of Fe(II) in the ground water and depletion of Fe(III) from the aquifer sediments suggested that the aromatic hydrocarbon oxidation was coupled to Fe(III) reduction. Geochemical modelling suggested that, in the portion of the aquifer closest to the hydrocarbon contamination, ca. half of the contaminant decomposition might be linked to Fe(III) reduction [7]. Since Fe(III) oxides have been depleted from the sediment near the source of contamination, the overall contribution of Fe(III) reduction to aromatic hydrocarbon decomposition within the whole of the anoxic plume may be greater than 50%.

In an aquifer contaminated with landfill leachate, most classes of organic contaminants monitored (monoaromatic hydrocarbons, substituted benzenes, napthalenes, chlorophenols) persisted as the ground water moved through zones in which methanogenesis and sulfate reduction were the terminal electron accepting process [74]. However, the contaminants were removed from the ground water as it entered a zone further downgradient where microbially-reducible Fe(III) oxides were still available and Fe(III) reduction was the predominant terminal electron accepting process. This suggested that the loss of contaminants was the result of contaminant oxidation coupled to Fe(III) reduction [74]. However, direct evidence that these contaminants were being oxidized to carbon dioxide by Fe(III)-reducing microorganisms was not provided.

Fe(III) reduction is an important process for the oxidation of naturally occurring organic matter in aquatic sediments [4,26,63]. Thus, there is the potential for Fe(III) reduction to be important in intrinsic bioremediation of organic contaminants in some sediments. Fe(III)-reducing enrichment cultures capable of degrading a variety of monoaromatic contaminant compounds were readily established with freshwater bottom sediments from the Potomac River [58]. However, the role of Fe(III) reduction in oxidizing contaminant organics in aquatic sediments has yet to be investigated in detail.

Dissimilatory Fe(III) reduction may also affect the fate of some organic contaminants through an indirect, non-enzymatic mechanism. The reduction of Fe(III) oxides by dissimilatory chloronitrobenzene to 4-chloroaniline [44]. It has been proposed that this type of reaction may account for the general nitroreductive capacity of anoxic soils and sediments [44].

Potential for engineered bioremediation of organic contaminants with Fe(III)-reducers

In some instances it may be possible to manipulate the activity of Fe(III)-reducing microorganisms in subsurface environments in order to accelerate the rate of organic contaminant degradation. For example, the activity of Fe(III)-reducing microorganisms can be stimulated by increasing the availability of Fe(III) for microbial Fe(III) reduction [72]. The Fe(III) in aquifer sediments is primarily in the form of insoluble Fe(III) oxides. In culture, Fe(III)-reducing microorganisms reduce soluble, chelated forms of Fe(III) much faster than insoluble Fe(III) oxides [60]. Fe(III) reduction was stimulated when the Fe(III) ligand, nitrilotriacetic acid (NTA), was added to sediments from a petroleum-contaminated aquifer in which Fe(III) reduction was the terminal electron accepting process. Stimulation of Fe(III) reduction enhanced the degradation of both toluene and benzene. Studies with ^{14}C-labelled toluene and benzene indicated that the compounds were being completely oxidized to carbon dioxide, presumably with Fe(III) as the electron acceptor. After an adaptation period, the rates of benzene and toluene degradation in the anoxic NTA-amended sediments were comparable to rates of degradation that have previously been observed in oxic aquifer material. This was surprising, because although microorganisms have been shown to degrade aromatic hydrocarbons under anoxic conditions [19,33,41,55,114], the rates of anoxic degradation were much slower than under oxic conditions. Aromatic hydrocarbons, especially highly toxic benzene, tend to persist in anoxic ground water [1,17,38,57,80,100,109].

These results demonstrate that O_2 is not necessary for rapid degradation of benzene in ground water. This may be useful information because the introduction of O_2 into ground water to stimulate bioremediation of petroleum contaminants can be technically difficult and expensive [57,80,82,100,109]. The addition of Fe(III) chelates to contaminated aquifers which still contain Fe(III) oxides should be relatively simple. Furthermore, it was found that the addition of soluble chelated Fe(III) could stimulate aromatic hydrocarbon degradation in contaminated aquifer material from which Fe(III) oxides had been depleted [72]. Thus, the stimulation of microbial Fe(III) reduction by enhancing the availability of Fe(III) shows potential as a novel approach to engineered bioremediation of petroleum-contaminated ground waters.

The finding that dissimilatory Fe(III) reduction can be important in the intrinsic bioremediation of organic contaminants and that this process could be manipulated to enhance contaminant degradation emphasizes the need to learn more about this relatively unexplored process. The metabolism of toluene by *Geobacter metallireducens* [62,67] is the only example of a dissimilatory Fe(III)-reducing microorganism in

isms responsible and their mode of metabolism are completely unknown.

Microbial reduction of toxic metals and metalloids

With the possible exception of Mn(IV) which is generally present at levels about 10-fold less than Fe(III) in soils, the concentrations of metals other than Fe(III) are too low for them to serve as significant oxidants for organic matter in sedimentary environments. However, microbial reduction of these less abundant metals can greatly affect their fate and mobility.

REDUCTIVE PRECIPITATION OF URANIUM

Irrigation practices [22,113], the mining and processing of uranium [75], and natural phenomena [104] can result in undesirably high concentrations of dissolved uranium in waters and waste streams. This dissolved uranium is generally in the form of U(VI) carbonate complexes [64]. Although U(VI) is highly soluble in most natural waters, U(IV) is highly insoluble [56]. Therefore, microbial reduction of U(VI) to U(IV) (reaction 2, Table 1) could potentially be used to precipitate uranium from contaminated waters and waste streams.

Several microorganisms are known to enzymatically reduce U(VI). These include *Geobacter metallireducens* and *Shewanella putrefaciens* which can conserve energy to support growth by coupling the oxidation of acetate (*G. metallireducens*) or H_2 (*S. putrefaciens*) to the reduction of U(VI) [68]. Several *Desulfovibrio* species also reduce U(VI) but do not conserve energy to support growth from this metabolism [65,69,71]. To date, evaluation of the practical use of microbial U(VI) reduction has focused on the *Desulfovibrio* species because they are easy to mass culture and can be stored aerobically in a freeze-dried state without losing their capacity for U(VI) reduction [64].

Microbial U(VI) reduction effectively precipitated uranium from solution under defined conditions in which the U(VI) was present in the form of a U(VI)-carbonate complex. Both *G. metallireducens* and *D. desulfuricans* rapidly converted high concentrations (ca. 1 mM) of U(VI) to U(IV) and precipitated all of the uranium from solution within several hours [40,64,65]. With both organisms, the U(IV) precipitate was all extracellular and was in the form of the mineral uraninite (UO_2). The uraninite readily settled to the bottom of the incubation vessels or could be removed with filtration. Alternatively, the U(VI)-reducing microorganisms could be maintained separately from the bulk of the contaminated water by placing the U(VI)-reducers within a dialysis sac [64]. The U(VI) diffused into the sac, the U(VI)-reducers converted the U(VI) to U(IV), and the U(IV) precipitated in the bottom of the sac. Of a wide variety of potentially inhibiting anions and cations that were evaluated, only high concentrations (>20 μM) of copper inhibited U(VI) reduction.

D. desulfuricans readily removed uranium from a variety of contaminated waters [64]. These included acidic (pH 4.0) and pH-neutral uranium mine drainage waters and uranium-contaminated groundwaters from the Department of Energy's Hanford site located near Richland, Washington. Uranium was also precipitated from the soil washings of a variety of uranium-contaminated soils [92]. The discovery that the c_3 cytochrome is the U(VI) reductase in *Desulfovibrio vulgaris* suggests the possibility of developing cell-free fixed enzyme systems and/or engineered organisms with enhanced U(VI)-reducing capacity for treating uranium-contaminated waters [71].

Microbial U(VI) reduction offers several advantages over other technologies for uranium removal such as ion exchange and biosorption [64]. Advantages include: 1) the ability to precipitate uranium from U(VI)-carbonate complexes; 2) the recovery of uranium in a highly concentrated and pure form; 3) high uranium removal per amount of biomass; 4) the potential to simultaneously treat organic contaminants and uranium by using the organic as an electron donor for U(VI) reduction; and 5) the potential for *in situ* remediation of groundwater. However, for small scale applications such as the removal of uranium from drinking water supplies for homes, technically less complex methods such as ion exchange resins [104] are likely to be more practical [64].

REDUCTIVE PRECIPITATION OF CHROMIUM

Manufacturing processes, domestic wastewater, and the dumping of sewage sludge are major sources of chromium contamination [28,81,91]. Cr(VI) and Cr(III) are the principal forms of chromium found in natural waters [91,96]. Cr(VI) is highly soluble and toxic whereas Cr(III) is much less toxic and tends to form insoluble hydroxides [81,91,96]. Cr(III) is also quickly immobilized in sediments by adsorption whereas Cr(VI) is not [81]. Therefore, reduction of Cr(VI) to Cr(III) (reaction 3, Table 1) is a potentially useful process for the remediation of chromium-contaminated waters and waste streams.

A wide variety of microorganisms can enzymatically reduce Cr(VI) to Cr(III) [61]. In those instances in which the mechanisms for Cr(VI) have been investigated, it is clear that the Cr(VI) reduction is an enzymatically catalyzed reaction [20,54,111,112]. Cr(VI) reduction may be a fortuitous reaction that is catalyzed by enzymes that have other physiological functions [27,50]. Energy conservation linked to Cr(VI) reduction has never been conclusively demonstrated [61].

Although all studies on microbial Cr(VI) reduction appear to have been conducted with the potential of microbial Cr(VI) reduction as a remediation tool in mind, the applied aspects of this process have been most extensively studied in *Enterobacter cloacae* strain HO1. Initial studies with *E. cloacae* demonstrated that high concentrations of Cr(VI) (as high as 10 mM) could be rapidly reduced, but the Cr(III) was difficult to remove from the culture [53]. For example, only ca. 30% of the Cr(III) that was produced from Cr(VI) reduction could be removed with centrifugation. Most of the soluble chromium could be removed from solution with Cr(VI) reduction if the cells of *E. cloacae* were placed on one side of an anion-exchange membrane which permitted CrO_4^- but not Cr^{3+} to pass through [52]. With this method, *E. cloacae* could reduce an initial concentration of 1 mM Cr(VI) down to ca. 0.04 mM dissolved chromium within 55 h.

However, the successful removal of dissolved chromium

during growth in heterotrophic growth medium could not be matched in contaminated waters. Cr(VI) was not reduced when a cell suspension of *E. cloacae* was added directly to a chromate-containing industrial effluent [85]. The Cr(VI) could be reduced if the effluent was diluted at least two-fold and supplemented with a nutrient solution containing peptone, meat extract, urea, and phosphate [85]. It was speculated that heavy metals were responsible for the inhibition of Cr(VI) reduction in the undiluted effluent. Further testing of heavy metal toxicity indicated that the following metal ions inhibited Cr(VI) reduction: $Hg^{2+} > Ag^{+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+}$ with Hg^{2+} inhibiting Cr(VI) reduction at concentrations as low as 1 μM [43]. Cr(VI) was not reduced in an industrial effluent that contained Cu^{2+}(390 μM), Mn^{2+}(480 μM) and Zn^{2+}(0.3 μM) [43]. Even when the heavy metals were removed, only about 40% of the Cr(VI) could be reduced. The residual inhibition of Cr(VI) reduction was attributed to high sulfate concentrations (60 mM) in the waste as similar concentrations of sulfate added to nutrient medium also inhibited Cr(VI) reduction by about 50%. These studies indicate that further investigation is required before *E. cloacae* can be applied to the remediation of real-world Cr(VI) contamination.

Desulfovibrio vulgaris might be more useful than *E. cloacae* for chromate bioremediation because sulfate and a variety of metals had no effect on Cr(VI) reduction by *D. vulgaris* [66]. An additional benefit was that *D. vulgaris* could reduce Cr(VI) in a simple mineral medium with H_2 serving as the electron donor. It did not need the rich heterotrophic medium required by *E. cloacae*. Freeze-dried cells of *D. vulgaris* that had been stored at room temperature could be used for Cr(VI) reduction. As with U(VI) reduction, the c_3 cytochrome functions as a Cr(VI) reductase in *D. vulgaris* [66].

In another study investigating the potential practical application of microbial Cr(VI) reduction, a mixed microbial community that was established as a biofilm on rotating plastic disks readily reduced Cr(VI) in a synthetic waste water [28]. Cr(VI) concentrations of as high as 200 mg L^{-1} were effectively treated to levels of 2–3 mg L^{-1} in the final effluent. The microorganisms responsible for the Cr(VI) reduction were not determined.

It seems doubtful that enzymatic Cr(VI) reduction would be easier and cheaper than non-enzymatic methods for treating waste waters. For example, Fe(II) rapidly reduces Cr(VI) at circumneutral pH, even under aerobic conditions, and the Cr(III)-Fe(III) hydroxides that form maintain dissolved chromium concentrations below the 10^{-6} M drinking water limit [32]. However, enzymatic Cr(VI) reduction may have some advantages over a non-enzymatic process for the *in situ* immobilization of chromium contamination. For example, a proposed *in situ* technique for preventing Cr(VI) migration is to form geochemical barriers of inorganic reductants such as pyrite or other Fe(II)-containing minerals [91]. However, the long-term *in situ* reduction of Cr(VI) with inorganic reductants such as Fe(II) and sulfide would require anoxic conditions to be effective. Generating and maintaining anoxic conditions may be technically difficult, especially in unsaturated soils. Furthermore, the generation of anaerobic conditions in ground water may result in other contamination problems such as the release of other trace metals. A number of Cr(VI)-reducing microorganisms reduce Cr(VI) aerobically [20,46,50]. Thus, if a technique was devised to stimulate Cr(VI) reduction in such aerobic Cr(VI)-reducing microorganisms this might be a useful strategy for *in situ* immobilization.

Enzymatic treatment of Cr(VI) might also be advantageous in mixed-waste situations where organic contaminants and Cr(VI) could be treated simultaneously by coupling the oxidation of the organic contaminants to the reduction of Cr(VI). Furthermore, when there are other metal contaminants such as uranium that are not easily chemically reduced (see above), it might be practical to use metal-reducing microorganisms to reduce Cr(VI) and the other metals simultaneously.

REDUCTIVE PRECIPITATION OF SELENIUM

Selenium contamination is associated with metal refining [83], fly ash waste [2], and agricultural drainage waters in the Western United States, most notably the highly publicized Kesterson National Wildlife Refuge [94]. The predominant redox states of selenium in natural environments are Se(VI) (selenate, SeO_4^{2-}), Se(IV) (selenite, SeO_3^{2-}), Se(0) (elemental selenium), and Se(-II) (selenide) [30]. The first three of these can potentially serve as electron acceptors for microbial metabolism. The reduction of highly soluble selenate to insoluble elemental selenium (reaction 4, Table 1) is a potential mechanism to remove selenate from contaminated waters.

The capacity to reduce selenate to elemental selenium is wide-spread in various genera of bacteria [18,25,51,79], but microorganisms with the capacity to conserve energy to support growth from selenate reduction have only recently been described. Two of these organisms, strains SES-1 [88] and SES-3 [87,106] reduce selenate to elemental selenium. Another, *Thauera selenatis* only reduces selenate to selenite [78]. However, many organisms reduce selenite to elemental selenium [30,87] and *T. selenatis* can be combined with a selenite reducer to produce elemental selenium [77].

Microbial reduction of selenate and selenite to insoluble elemental selenium has been observed in a variety of soils and aquatic sediments [31,79,88,105]. This metabolism intrinsically bioremediates the agricultural drainage water that is collected in evaporation ponds and may remove selenate from ground waters [87,89,90]. Estimates of *in situ* selenate reduction in the sediments of an evaporation pond suggested that microbial selenate reduction could remove all of the selenate in the overlying water within several months [89].

Furthermore, it is possible to engineer conditions to enhance microbial selenate reduction. For example, when exposed soil at Kesterson Reservoir was flooded with selenium-free water in order to generate anoxic conditions, 66–110% of the dissolved selenium that had been present in the upper 1.22 m of soil was immobilized [59]. A limitation to this technique is that bottom-feeding organisms may bring some of the immobilized elemental selenium back into the food chain [73]. Furthermore, the soils must be maintained in an anoxic state because once the soils are oxidized the sequestered selenium may become highly mobile [3].

Selenate may also be treated in *ex situ* treatment processes

in which selenate-contaminated waters are passed through soil columns or bio-reactors which contain selenate-reducing microorganisms [5,37,51,76,89]. A cost evaluation of the use of selenate- and selenite-reducing microorganisms to remove selenium from waste waters concluded that the microbial process could potentially be cheaper than chemical reduction if a nutrient cheaper than the peptone that was used to culture the organisms could be found [5]. The requirement for a cheap carbon and energy source for selenate reducers might be met by first growing algae in the nutrient-rich water [37,89]. This technique also serves to remove the nitrate which can inhibit selenate reduction by some organisms. However, nitrate is not always a concern as some selenate reducers continue to reduce selenate despite the presence of nitrate in contaminated waters [76]. Oremland [87] raises the point of whether the value of the irrigated crops justifies the cost of water treatment and the water consumed by irrigation.

REDUCTIVE PRECIPITATION OF LEAD

The reduction of soluble Pb(II) to insoluble Pb(0) (reaction 5, Table 1) is a potential mechanism for removing lead from solution. When *Pseudomonas maltophilia* was grown aerobically in an organic-rich medium in the presence of 1 mM lead acetate, dissolved lead decreased over time and there was an accumulation of a gray-black, lead-containing colloid [99]. The colloids had a diameter of 175 nm and had a strongly negative zeta potential. The colloidal lead aggregated and settled out of solution. Several techniques other than settling by gravity for removing the lead colloid from water are being investigated [99]. However, whether the lead precipitation was actually the result of reduction of Pb(II) to Pb(0) was not demonstrated.

REDUCTIVE PRECIPITATION OF TECHNETIUM

Technetium is a long-lived (half-life, 2.15×10^5 years) radioactive contaminant in the environment that is a by product of fission reactions during atomic explosions and in nuclear power stations [110]. The oxidized form of technetium is the highly soluble pertechnetate ion (TcO_4^-; Tc(VII)) [110]. Tc(IV), the reduced form of technetium, is highly insoluble [110]. Thus, reduction of technetium (reaction 6, Table 1) can greatly reduce its mobility in the environment [102].

The role of microorganisms in enzymatically reducing technetium in the environment has not been adequately studied. There are several studies with pure cultures and enrichments that suggest that microorganisms may enzymatically reduce technetium [45,93]. There do not appear to have been any studies on the potential for using microorganisms to remove technetium from contaminated waters or waste streams. However, it seems possible that microbial technetium reduction could be used for bioremediation in a manner similar to that described for uranium [68].

REDUCTIVE VOLATILIZATION OF MERCURY

Mercury is the most common metal contaminant that is a human health concern [84]. A number of microorganisms, reduce ionic Hg(II) to Hg(0) (reaction 7, Table 1) during aerobic growth as a detoxification mechanism [23,97,107]. Whereas Hg(II) is highly soluble, Hg(0) is volatile and thus can be readily vented or extracted from contaminated environments or waste streams [42,84,108].

Removal of mercury through reductive volatilization of mercury may be an intrinsic remediation process in both aquatic and terrestrial environments [16,39,84]. Although some of this Hg(II) reduction is clearly microbiological, in some environments non-biological mechanisms may also be important [12,14,16,95]. The microbial community typically responds to mercury contamination with an enhanced capacity for Hg(II) reduction [8,10,12–16,86,98].

It may be possible to stimulate rates of microbial Hg(II) reduction as an engineering approach to *in situ* bioremediation of contaminated soils and water [16,39,84]. This could potentially be accomplished by the addition of Hg(II) reducers, stimulating the growth of indigenous populations, amplifying the number of *mer* operons in indigenous microorganisms, or by increasing the percentage of indigenous *mer* operons that are expressed. However, a limitation on the use of microbial Hg(II) reduction for *in situ* bioremediation is that most of the Hg(II) is typically unavailable for microbial reduction [16,95,103]. Another concern for *in situ* treatment is that, unless special recovery systems are developed, the mercury removed from the contaminant site will be transported in the atmosphere and deposited elsewhere.

The potential for *ex situ* remediation of mercury-contaminated waters and waste streams has also been investigated. For example, mercury that was chemically precipitated from liquid mining and chemical wastes and then redissolved in nutrient medium could be volatilized with the mercury-resistant microorganism *Pseudomonas* K62 [108]. The chemical pretreatment was necessary because salts in the industrial waste could have inhibited Hg(II) reduction. The chemical pretreatment that was used in precipitating the mercury was expensive and a cheaper alternative method would be required for microbial Hg(II) reduction to be used on an industrial scale.

Hansen and coworkers [42] found that a mixed culture reactor incubated at 37 °C and fed mercury-amended raw sewage at an influent detention time of 1 day, continuously removed 88% of the 70 mg L^{-1} Hg(II) in the influent. If the effluent was allowed to incubate an additional day at room temperature, then 98% removal of mercury was achieved. The fate of the mercury was not determined but it was assumed to be removed in the effluent air stream. It was considered that the Hg(0) in the effluent air might be recovered through condensation.

In a similar manner, a pure culture of *Pseudomonas putida* FB1 removed mercury when grown under continuous culture conditions on an organic-rich medium [9]. Effluent mercury concentrations could be maintained below the upper legal limit for mercury in liquid wastes.

Immobilized Hg(II)-reducing microorganisms in small laboratory columns effectively removed mercury from solution, depositing elemental mercury in particles about 1–5 μm in diameter outside the cells [24,36]. The effluent from the column typically had mercury concentrations of less than

50 $\mu g\ L^{-1}$. Mercury retention was improved when pure strains were used instead of a natural consortia. In a stirred tank configuration the elemental mercury was released into the atmosphere. Mercury reductase activity could be enhanced through genetic engineering to generate strains which constitutively produced Hg(II)-reductase [24]. Furthermore, organisms with enhanced capacity for cleaving mercury from organomercurial pollutants and then reducing the released Hg(II) to Hg(0) can be engineered [47].

Several studies have suggested that microbial Hg(II) reduction might be a better method of mercury removal than chemical methods which can be expensive and result in the generation of a mercury-containing sludge [9,42]. However, side by side comparisons between biological and chemical methods have not been presented and microbial Hg(II) reduction has only been tested at the bench scale. Thus, although the microbial mechanisms and genetics for microbial Hg(II) reduction are better understood than for any other type of microbial metal reduction, it appears that even for this process, substantial additional research will be required before it will be apparent whether microbial metal reduction can be used as a bioremediation tool.

CONCLUSION

The studies summarized here demonstrate that microbial metal reduction has the potential to be a useful technique for the biorestoration of environments contaminated with organics and/or metals. As recently reviewed [61], microorganisms may enzymatically reduce other metals such as vanadium, molybdenum, copper, gold, and silver. These redox changes can affect the solubility of these metals as well and could potentially be applied in bioremediation strategies. In comparison with the vast amount of research that has been done on the aerobic degradation of organic contaminants, there has been relatively little investigation into the use of microbial metal reduction for bioremediation. More basic research into this potentially useful type of metabolism is required, as are attempts to try to scale-up techniques that have shown promise on a laboratory scale.

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