The European Journal of Mineral Processing and Environmental Protection
Vol.3, No.1, 1303-0868, 2003, pp. 58-66

Review

# Basic concepts on heavy metal soil bioremediation

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Received 30 June 2002; accepted 12 January 2003

ABSTRACT

The utilization of organisms, primarily microbes, to clean up contaminated soils, aquifers, sludges, residues, and air, known as "bioremediation", is a rapidly changing and expanding area of environmental biotechnology, that offers a potentially more effective and economical clean-up technique than conventional physicochemical methods. Although it is certain that up to now the technologies employed are not technically complex, considerable experience and expertise is required to design and implement a successful bioremediation program. As a matter of fact, and since bioremediation frequently addresses multiphasic, heterogenous environments (i.e., soils), successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology, and chemistry. The bio-enthusiasm of the early years that followed the initial promising research results and inspired the creation of many remediation companies has ended in a more realistic and sometimes even sceptical view of bioremediation since it has now become clear that results obtained in the laboratory do not necessarily indicate what may happen actually in the field, since it is not possible to simulate all the changing conditions of a real situation.

Most traditional remediation methods do not provide acceptable solutions for the removal of metals from soils. Microorganisms that use metals as terminal electron acceptors, or reduce metals as a detoxification mechanism can be used for the removal of metals from contaminated environments. In some cases, phytoextraction of metals is a cost-effective approach that uses metal-accumulating plants to clean up metal polluted soils. 

Keywords: Soil; Metals; Bioremediation; Phytoremediation; Environmental

## 1. INTRODUCTION

The quality of life on Earth is linked inextricably to the overall quality of the environment. It is very difficult to define soil quality, as soil composition can vary from place to place. Soil quality is concerned with more than the soil's constituents and composition, but how it functions in a specific environment. The major functions of a soil are generally recognized to include the ability to protect water and air quality, the ability to sustain plant and animal productivity, and the ability to promote human health (Doran and Parkin, 1994; Chen and Mulla, 1999).

The release of contaminants into the environment by human activities has increased enormously over the past several decades. In fact, although a few decades ago, man's greatest challenge resided in speeding up the industrialization process, today man attempts to find ways to deal with the growing industrialization and the associated problems (Thassitou and Arvanitoyannis, 2001). The relatively sudden introduction of pollutants into the recipient ecosystems has clearly overwhelmed their self-cleaning capacity and, as a consequence, resulted in the accumulation of pollutants. Soil pollution has recently been attracting

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considerable public attention since the magnitude of the problem in our soils calls for immediate action.

The large-scale production of a variety of chemical compounds, such as organic solvents, fuels and fuel additives, pesticides, plasticizers, pigments, dyes, plastics and chemical feedstocks, has caused global deterioration of environmental quality (Garbisu and Alkorta, 1997; Iwamoto and Nasu, 2001).

Contaminated soils are a common environmental problem all over the world. The various countries confronted with contaminated soil differ considerably in awareness of the problem and in the policies and the technologies to tackle it (Rulkens *et al.*, 1998). Nonetheless, intensive exchange of experiences gained with the management and remediation of polluted soils is taking place among the various countries.

As a matter of fact, increasingly widespread pollution has caused vast areas of land to become non-arable and hazardous for both wildlife and human populations. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today (Vidali, 2001). Unfortunately, the enormous costs associated with the removal of pollutants from soils by means of traditional physicochemical methods have been encouraging companies to ignore the problem (Alkorta and Garbisu, 2001).

In addition to minimizing the impact of future incidents by means of controlling soil pollution input (developing a long-term perspective of pollution amelioration measures that focus on slowing the rate of pollution increase), it is imperative to deploy innovative technologies which could economically remediate toxic wastes adversely impacting our environment, thereby reducing the threat to human health and the environment (Garbisu and Alkorta, 1997).

In the last few years, disquiet among ordinary people has grown and the public is now strongly demanding clean-up measures to be urgently introduced. In this context, governmental recognition of the accumulating hazards has resulted in legislative restrictions on uncontrolled discharges of wastes and actions mandating environmental restoration of hazardous waste sites. This recent environmental awareness has highlighted the need for new technologies for the treatment of these wastes.

## 2. WHAT IS BIOREMEDIATION?

Traditional physicochemical processes for remediation of soil polluted sites are expensive and often do not permanently alleviate the pollution hazard. The most common conventional techniques used for remediation are: (i) excavation and disposal to a landfill, and (ii) to cap and contain the contaminated areas of a site.

Apart from the fact that it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material, the first method simply moves the contamination elsewhere (with the possibility of creating risks during the excavation, handling, and transport of hazardous material), while the second is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers (Vidali, 2001). Other methods such as incineration lack public acceptance since they can increase the exposure to contaminants of both the workers at the site and nearby residents. Some other techniques that are in various stages of development are the following: extraction of pollutants with organic solvents or $CO_2$, oxidation of organic pollutants under subcritical or supercritical conditions, vitrification, electroreclamation, dehalogenation of chlorinated organic compounds using an alkali polyethylene glycol, chemical reduction or oxidation of contaminants, steam stripping, plasma torch techniques, microwave heating, solidification/stabilisation, and so on (Rulkens *et al.*, 1998).

Bioremediation is a general concept that includes all those processes and actions that take place in order to biotransform an environment, already altered by contaminants, to its original status. Bioremediation uses primarily microorganisms or microbial processes to degrade and transform environmental contaminants into harmless or less toxic forms. Although strictly speaking, bioremediation is the use of "organisms" to degrade pollutants, it is mainly concerned with the use of "microorganisms". In this context, phytoremediation, defined as the use of green plants to remove environmental contaminants or to render them harmless (Cunningham and Berti, 1993; Raskin *et al.*, 1994, 1997; Salt *et al.*, 1995, 1998), is recently being considered as a highly promising technology for the remediation of polluted sites. This topic of phytoremediation has recently been reviewed by the authors in more detail elsewhere (Alkorta and Garbisu, 2001; Garbisu and Alkorta, 2001; Garbisu *et al.*, 2002).

## 3. BIOREMEDIATION OF HEAVY METALS

The term "heavy metal" is arbitrary and imprecise. Some authors (Raskin *et al.*, 1994), for the sake of simplicity, defined "heavy metal" as any element that has metallic properties (ductility, conductivity, density, stability as cations, ligand specificity, etc.) and an atomic number greater than 20. A more biologically relevant classification of metals based on ligand-forming properties has been proposed (Nieboer and Richardson, 1980).

Several metals are essential for biological systems and must be present in a certain concentration range. In fact, they provide essential cofactors for metalloproteins and enzymes and, consequently, too low concentrations lead to a decrease in metabolic activity. At high concentrations, metals can act in a deleterious manner by blocking essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecules (Collins and Stotzky, 1989). Besides, they are toxic for both higher organisms and microorganisms. Nonessential metals are tolerated at very low concentrations and inhibit metabolic activity at higher concentrations.

As a result of their toxicity, the presence of heavy metals in polluted sites can interfere with remediation processes. The progressive accumulation of metals may inhibit the degradation of organic pollutants or of humic substances in the environment. This problem can be solved by an increase of the heavy metal resistance of the bioremediating system. In this context, when degradative microbial populations are inoculated in a contaminated site they must possess both degradative enzymes for the target pollutant(s) as well as resistance to relevant heavy metals present in the area.

Heavy metals are present in soils and aqueous streams as both natural components or as a result of human activity (i.e., metal-rich mine tailings, metal smelting, electroplating, gas exhaust, energy and fuel production, downwash from power lines, intensive agriculture, sludge dumping, etc.) (Raskin *et al.*, 1994).

Metals are somewhat unique in that they do not undergo either chemically or biologically induced degradation that can alter or reduce their toxicity over time (Knox *et al.*, 2000). Microorganisms are not alchemists; no matter how a microorganism acts upon a toxic metal, the metal is not destroyed (Lovley and Lloyd, 2000). That is to say, heavy metals cannot be destroyed biologically (no "degradation", change in the nuclear structure of the element, occurs) but are only transformed from one oxidation state or organic complex to another. As a consequence of the alteration of its oxidation state, the metal may become either: (i) more water soluble and be removed by leaching, (ii) inherently less toxic, (iii) less water soluble so that it precipitates and then becomes less bioavailable or removed from the contaminated site, or (iv) volatilized and removed from the polluted area (Garbisu and Alkorta, 1997).

Metals in soil need to be removed from the matrix by solubilization in a liquid phase. Afterwards, they can be concentrated in the desolubilization phase (Diels *et al.*, 1999). Many microorganisms produce siderophores, iron complexing molecules, some of which have high affinity for heavy metals. In *Pseudomonas aeruginosa* and *Alcaligenes eutrophus*, siderophore synthesis was also induced by heavy metals in the presence of high iron concentrations (Höfte *et al.*, 1994; Gilis *et al.*, 1996).

Microorganisms can detoxify metals by valence transformation, extracellular chemical precipitation, or volatilization. They can enzymatically reduce some metals in metabolic processes that are not related to metal assimilation (Lovley, 1993). Several bacteria couple the oxidation of simple organic acids and alcohols, hydrogen, or aromatic compounds, to the reduction of Fe(III) or Mn(IV). Bacteria that use U(VI) as a terminal electron acceptor may be useful for uranium bioremediation (Lovley, 1993). The reduction of the toxic selenate and selenite to the much less toxic elemental selenium may be exploited for selenium bioremediation (Garbisu *et al.*, 1995a, b, 1997a, b). Biomethylation to yield volatile derivatives such as dimethylselenide or trimethylarsine is a well-known phenomenon catalyzed by several bacteria, algae and fungi (White *et al.*, 1997). Several bacteria have been reported to reduce hexavalent chromium that is toxic and mutagenic, to its trivalent form that is less toxic (Garbisu *et al.*, 1997c, 1998; Ishibashi *et al.*, 1990; Wang *et al.*, 1989). Bioprecipitation by sulfate-reducing bacteria that convert sulfate to hydrogen sulfide, which, in turn, reacts with heavy metals to form insoluble metal sulfides such as zinc sulfide and cadmium sulfide has been reported in some bacteria (White *et al.*, 1998; Iwamoto and Nasu, 2001).

However, the only *in situ* strategy that employs microorganisms and actually removes a metal contaminant from soil is microbial reduction of soluble mercuric ion, Hg(II), to volatile metallic mercury, Hg(0) (Hobman and Brown, 1997). The reduced Hg(0) can then flux out of the contaminated area and be diluted in the atmosphere (Lovley and Lloyd, 2000). Unfortunately, microorganisms do not readily volatilize most other toxic metals.

Microorganisms can also enzymatically reduce other metals such as technetium, vanadium, molybdenum, gold, silver, etc. but these processes have not been studied extensively (Lovley, 1993).

Valls *et al.* (2000) have reported on the addition of specially engineered *Ralstonia eutropha*, a natural inhabitant of soil, to sequester metals from polluted soils. Although the toxic metals remain in the soil, once they are bound to the microorganisms, they become less bioavailable. It is well known that bacteria can bind metals to their cell surfaces, but, unfortunately, their natural binding capacity is generally insufficient to significantly mitigate metal contamination. Although microorganisms have only rarely been found to produce metallothioneins (i.e., small cysteine-rich proteins that bind heavy metals) (Stillman, 1995), Valls *et al.* (2000) found that the mouse gene encoding metallothionein production could be expressed in *R. eutropha*. The studies on the ability of this bacterium to lower the toxicity of cadmium have so far been promising.

The major limitation of these remediation methods is that, although the metals are concentrated or converted into less toxic forms, they are still present in the soil and need to be effectively extracted from it. Afterwards, the concentrated product can be dumped in a controlled way or recycled for metal recovery (Diels *et al.*, 1999). In this context, the phytoremediation of heavy metals from soils, known as phytoextraction, that uses the uptake capabilities of plants, represents one of the largest economic opportunities for phytoremediation. Plants can accumulate metals that are essential for growth and development (such as Cu, Mn, Fe, Zn, Mo, and possibly Ni) and also some that have no known biological function (Cd, Cr, Pb, Co, Ag, Se, Hg) (Baker and Brooks, 1989; Brooks, 1998; Raskin *et al.*, 1994). In this context, plants have been described as solar-driven pumping stations (Cunningham *et al.*, 1995) which can actually remove these contaminants from the environment.

Most existing physicochemical remediation technologies are meant primarily for intensive *in situ* or *ex situ* treatment of relatively highly polluted sites, and thus are not very suitable for the remediation of vast, diffusely polluted areas where pollutants occur only at relatively low concentrations and superficially (Garbisu and Alkorta, 2001; Rulkens *et al.*, 1998). Phytoremediation is best suited for the remediation of these diffusely polluted areas at much lower costs than other methods (Garbisu and Alkorta, 2001). Although it is true that this remediation procedures can take rather long, more and more often, this is not considered a problem as far as the costs are lower and the risks posed to human populations and ecosystems are acceptable (Rulkens *et al.*, 1998).

As a general rule, readily bioavailable metals for plant uptake include Cd, Ni, Zn, As, Se, and Cu. Moderately bioavailable metals are Co, Mn, and Fe; while Pb, Cr, and U are not very bioavailable (Miller, 1996).

As pointed out in the excellent review by Salt *et al.* (1998), there are, at present, two strategies of phytoextraction: (i) continuous phytoextraction, using hyperaccumulators, and (ii) chelate-assisted or induced phytoextraction. The first strategy of metal phytoextraction depends on the natural ability of some plants to accumulate, translocate and resist high amounts of metals over the complete growth cycle. Hyperaccumulators are the most suitable plants since they can accumulate 10-500 times higher levels of elements than crops (Chaney *et al.*, 1997). The *Brassicaceae* family, to which many hyperaccumulator species belong, is also interesting because the high content of thyocianates makes this species non-palatable to animals – a characteristic that is likely to reduce the chances of bioaccumulation of metals in the food chain during phytoextraction programs (Navari-Izzo and Quartacci, 2001). This possibility of contaminating the food chain is one of the main problems associated with phytoextraction techniques.

Chelate-assisted or induced phytoextraction is based on the fact that the application of metal chelates to the soil significantly enhances metal accumulation by plants. Under many circumstances, in the soil and depending on the metal itself, it is common to find cases of low bioavailability, preventing the remediation process (a large proportion of many metals remains sorbed to solid soil constituents).

Fortunately, the discovery that the application of certain chelates to the soil increases the translocation of heavy metals from soil into the shoots has opened a wide range of possibilities for this field of metal phytoextraction (Blaylock *et al.*, 1997). However, the application of synthetic chelates to the soil must be done carefully because of their potential toxicity.

One indication of acceptability of a technique is previous successful applications on similar sites. Because it is a relatively new technology, phytoremediation does not have a long history of completed cleanups. In any case, the U. S. Environmental Protection Agency reported a list of 180 sites (many of them polluted with heavy metals) where this technology was applied or was being field-tested (EPA, 2000). Small-scale field trials are demonstrating the feasibility of the phytoextraction approach.

## 4. ECONOMIC CONSIDERATIONS OF PHYTOREMEDIATION

Bioremediation of polluted sites can reduce clean-up costs since it treats contamination in place, harnesses natural processes and reduces environmental stresses (Chapelle, 1997). In fact, most of the cost of conventional physicochemical clean-up technologies is associated with physically removing and disposing of contaminated soils. Besides, at some sites, natural processes can remove or contain contaminants without human intervention and this natural attenuation leads to substantial cost savings. Likewise, and since bioremediation methods minimize site disturbance compared with conventional clean-up technologies, post-clean-up costs can be substantially reduced.

Remediation costs can be minimized, without jeopardizing effectiveness, by gaining a better understanding of remediation procedures and the various options available at the different stages in the process (Tripp, 1996). The first step is the proper analysis and identification of the contamination problem, both its exact nature and extent. The more complete this initial analysis the less likely that costly surprises will surface at a later stage. The next step is the development of a remediation plan which includes a feasibility analysis. This feasibility analysis should include such factors as type of soils, contaminated location, nature of contamination, amount of contaminated material, time required to remediate the site, time of year, use of remediation specialists, and so on.

In relation to phytoremediation, most likely the best choice for polluted soils containing relatively low concentrations of metals, it is important to emphasize that phytoremediation is an emerging technology and thus standard cost information is not readily available. In any case, Glass (1988) estimated that total system costs for some phytoremediation applications will be 50 to 80% lower than alternatives. Each application of plants will yield a separate performance evaluation including rate and extent of clean-up and cost.

The ability to develop cost comparisons and to estimate project costs needs to be determined on a site-specific basis. Two considerations influence the economics of phytoremediation: the potential for application and the cost comparison to conventional treatments (EPA, 2000). Whole system costs should include, among others, design costs (site characterization, work plan, report preparation, treatability and pilot testing), installation costs (site preparation, soil preparation, infrastructure, planting) and operating costs (maintenance and monitoring).

Blaylock *et al.* (1997) reported the estimated cost of cleaning up 1-acre of lead polluted soil. While for a conventional treatment such as excavate and landfill, the estimated costs were $500 (€460), for phytoremediation (extraction, harvest and disposal) the costs were $150-250 (€140-230) (50-65% savings).

Hypothetical cost comparisons have been carried out based on laboratory and pilot scale work and tend to reflect projected total project costs. Cunningham (1996) reported that the estimated 30-year costs for remediating a 12-acre lead site were $12,000,000 (€11,100,000) for excavating and disposal, $6,300,000 (€5,833,000) for soil washing, $600,000 (€555,000) for a soil cap, and $200,000 (€185,000) for phytoextraction.

Costs were estimated to be $60,000 (€55,000) to $100,000 (€92,500) using phytoextraction for remediation of 1-acre of 20-in-thick sandy loam compared to a minimum of $400,000 (€370,000) for just excavation and storage of this soil (Salt *et al.*, 1995).

## 5. DEVELOPMENTS IN MOLECULAR MICROBIAL ECOLOGY

Our current knowledge of changes in microbial communities during a bioremediation process is very limited and, consequently, the microbial community is still treated as a “black box” (Iwamoto and Nasu, 2001). This is mostly due to the fact that many environmental bacteria cannot yet be cultured by conventional laboratory techniques (Kogure *et al.*, 1979; Olsen and Bakken, 1987). Because of this limitation, the bioremediation often faces the difficulty of identifying the cause and developing measures in the case of failure remediation from a microbiological standpoint.

Fortunately, the recent advances in the field of molecular biological methods are helping us to study the structure and dynamics of microbial communities without bias introduced by cultivation. These molecular biological techniques are frequently used in microbial ecological studies.

Very briefly, since this paper does not intend to deal with this topic in detail, the molecular methods that can be used to study an *in situ* bioremediation process for the detection and monitoring of target bacteria are the following: (i) fluorescence *in situ* hybridization (FISH) with rRNA targeted oligonucleotide probes (Hahn *et al.*, 1992), and (ii) *in situ* PCR (Hodson *et al.*, 1995). Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA fragments has emerged as a powerful technique for monitoring changes in bacterial diversity (Muyzer *et al.*, 1993). Another method for the study of microbial community diversity is terminal restriction fragment length polymorphism (T-RFLP) (Liu *et al.*, 1997).

Thanks to all these novel techniques, microbiologists have now realized that natural microbial populations, including pollutant-degrading microorganisms, are much more diverse than those expected from the catalog of isolated microorganisms (Watanabe, 2001). Recent studies have applied molecular tools to the analysis of bacterial (Brim *et al.*, 1999; Sandaa *et al.*, 1999a) and archaeal populations (Sandaa *et al.*, 1999b) that are capable of surviving in metal-contaminated environments. The detoxification machineries that some of these organisms may have are considered useful for metal bioremediation.

## 6. CONCLUSIONS

Bioremediation is still an immature technology and needs to define its boundaries between promise and reality. It frequently addresses multiphasic, heterogenous environments (i.e., soils), and so successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology, and chemistry. The interdisciplinary approach is also required because of the complexity encountered in the type and extent of contamination and the social and legal issues relevant to most contaminated sites.

Through improved understanding of the ecology, physiology, evolution, biochemistry, and genetics of microorganisms, the prospect for successfully stimulating and exploiting microbial metabolism for environmental purposes appears very promising. Despite its limitations, the future of bioremediation appears bright as the advances in the diverse disciples that shape bioremediation are accelerating.

Progress in developing strategies for *in situ* microbial approaches to metals remediation has clearly lagged significantly behind the development of *in situ* bioremediation of organics. However, and since funding opportunities for research on *in situ* bioremediation of metals has increased dramatically in recent years, it seems likely that novel advances in this area will be forthcoming.

Small-scale field trials are demonstrating the feasibility of the phytoextraction approach. As a matter of fact, phytoextraction appears a very promising technology for the removal of metal pollutants from the environment and may be, at present, approaching comercialization. Phytoremediation methods are well suited for use at very large field sites where other methods of remediation are not cost effective or practicable.

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