Review paper

Factors limiting bioremediation technologies

R. Boopathy

Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310, USA

Abstract

The use of microorganisms to destroy, or reduce the concentration of, hazardous wastes on a contaminated site is called bioremediation. Such a biological treatment system has various applications, including, clean up of contaminated sites such as water, soils, sludges, and waste streams. The treatment of the Alaskan shoreline of Prince Williams Sound after the oil spill of Exxon Valdez in 1989 is one common example in which bioremediation methods got public attention. There are numerous other success stories of bioremediation in cleaning up chemical spills, leaking underground storage tanks of gasoline, and many toxic industrial effluents. This paper outlines the various factors, including scientific, non-scientific, and regulatory, that limit the use of bioremediation technologies. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Bioremediation; Biochemistry; Bioavailability; Biotreatment; In situ; Bioactivity

1. Introduction

Contamination of soils, groundwater, sediments, surface water, and air with hazardous and toxic chemicals is one of the major problems facing the industrialized world today. The national priority list currently contains over 1200 sites, with potential sites numbering over 32 000 (Baker and Herson, 1994). The need to remediate these sites has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal. Bioremediation, the use of microorganisms or microbial processes to degrade environmental contaminants, is among these new technologies. Bioremediation has numerous applications, including clean-up of ground water, soils, lagoons, sludges, and process-waste streams. Bioremediation has been used on very large-scale application, as demonstrated by the shore-line clean-up efforts in Prince William Sound, Alaska, after the Exxon Oil spill. Although the Alaska oil-spill clean-up represents the most extensive use of bioremediation on any one site, there have been many other successful application on smaller scale.

Bioremediation frequently must address multiphasic, heterogenous environments, such as soils in which the contaminant is present in association with the soil particles, dissolved in soil liquids, and in the soil atmosphere. Because of these complexities, successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology, and chemistry. In this paper, some of the obstacles, both scientific and non-scientific, to bioremediation are discussed.

2. Bioremediation methods

2.1. In situ and ex situ methods

Bioremediation technologies can be broadly classified as ex situ and in situ. Ex situ technologies are those treatments which involve the physical removal of the contaminated material for treatment process. In contrast, in situ techniques involve treatment of the contaminated material in place. Some of the examples of in situ and ex situ bioremediation are given below:

1. *Land farming*: Solid-phase treatment system for contaminated soils: may be done in situ or ex situ.
2. *Composting*: Aerobic, thermophilic treatment process in which contaminated material is mixed with a bulking agent; can be done using static piles or aerated piles.
3. *Bioreactors*: Biodegradation in a container or reactor; may be used to treat liquids or slurries.
4. *Bioventing*: Method of treating contaminated soils by drawing oxygen through the soil to stimulate microbial activity.
5. *Biofilters*: Use of microbial stripping columns to treat air emissions.

6. *Bioaugmentation*: Addition of bacterial cultures to a contaminated medium; frequently used in both in situ and ex situ systems.
7. *Biostimulation*: Stimulation of indigenous microbial populations in soils or ground water by providing necessary nutrients.
8. *Intrinsic bioremediation*: Unassisted bioremediation of contaminant; only regular monitoring is done.
9. *Pump and treat*: Pumping ground water to the surface, treating, and reinjecting.

2.2. Advantages and disadvantages of bioremediation

For bioremediation to be successful, the bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur. The right microbes are bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants. Bioremediation offers several advantages over conventional techniques such as land filling or incineration. Bioremediation can be done on site, is often less expensive and site disruption is minimal, it eliminates waste permanently, eliminates long-term liability, and has greater public acceptance, with regulatory encouragement, and it can be coupled with other physical or chemical treatment methods. Bioremediation has also its limitations. Some chemicals are not amenable to biodegradation, for instance, heavy metals, radionuclides and some chlorinated compounds. In some cases, microbial metabolism of contaminants may produce toxic metabolites. Bioremediation is a scientifically intensive procedure which must be tailored to the site-specific conditions, which means one has to do treatability studies on a small-scale before the actual clean-up of the sites. Some of the questions one has to answer before using bioremediation techniques are: is the contaminant biodegradable? is biodegradation occurring in the site naturally? are environmental conditions appropriate for biodegradation? if the waste does not completely biodegrade, where will it go? These questions can be answered by doing site characterization and also by treatability studies.

2.3. Physiology of biodegradative microbes

A bioremediation process is based on the activities of aerobic or anaerobic heterotrophic microorganisms. Microbial activity is affected by a number of physicochemical environmental parameters. The factors that directly impact on bioremediation are energy sources (electron donors), electron acceptors, nutrients, pH, temperature, and inhibitory substrates or metabolites. One of the primary distinctions between surface soils, vadose zone soils and groundwater sediments is the content of organic material. Surface soils, which typically receive regular inputs of organic material from plants, will have higher organic matter content. The high organic matter content is typically associated with high microbial numbers and a great diversity of microbial populations. The organic matter serves as a storehouse of carbon and energy as well as a source of other macronutrients such as nitrogen, phosphorous, and sulfur. Subsurface soils, and ground water sediments have lower levels of organic matter and thus lower microbial numbers and population diversity than surface soils (Adriaens and Hickey, 1993). Bacteria become more dominant in the microbial community with increasing depth in the soil profile as the numbers of other organisms such as fungi or actinomycetes decrease. This is attributed to the ability of bacteria to use alternative electron acceptors to oxygen. Other factors that control microbial populations are moisture content, dissolved oxygen, and temperature.

2.4. Metabolic processes

Primary metabolism of an organic compound has been defined as the use of the substrate as a source of carbon and energy. This substrate serves as an electron donor resulting in microbial growth. Application of co-metabolism to site-remediation of xenobiotics is required when the compound cannot serve as a source of carbon and energy by nature of the molecular structure, which does not induce the required catabolic enzymes. The term co-metabolism has been defined as the metabolism of a compound that does not serve as a source of carbon and energy or as an essential nutrient which can be achieved only in the presence of a primary (enzyme inducing) substrate.

Aerobic processes are characterized by metabolic activities involving oxygen as a reactant. Dioxygenases and monooxygenases are two of the primary enzymes employed by aerobic organisms during transformation and mineralization of xenobiotics. Anaerobic microbes take advantage of a range of electron acceptors, which, depending on their availability and the prevailing redox conditions, include nitrate, iron, manganese, sulfate, and carbon dioxide.

3. Scientific factors affecting bioremediation

3.1. Energy sources

One of the primary variables affecting the activity of bacteria is the ability and availability of reduced organic materials to serve as energy sources (Table 1). Whether a contaminant will serve as an effective energy source for an aerobic heterotrophic organism is a function of the average oxidation state of the carbon in the material. In general, higher oxidation states correspond to lower

Table 1
Major factors affecting bioremediation

Microbial
  Growth until critical biomass is reached
  Mutation and horizontal gene transfer
  Enzyme induction
  Enrichment of the capable microbial populations
  Production of toxic metabolites

Environmental
  Depletion of preferential substrates
  Lack of nutrients
  Inhibitory environmental conditions

Substrate
  Too low concentration of contaminants
  Chemical structure of contaminants
  Toxicity of contaminants
  Solubility of contaminants

Biological aerobic vs anaerobic process
  Oxidation/reduction potential
  Availability of electron acceptors
  Microbial population present in the site

Growth substrate vs co-metabolism
  Type of contaminants
  Concentration
  Alternate carbon source present
  Microbial interaction (competition, succession, and predation)

Physico-chemical bioavailability of pollutants
  Equilibrium sorption
  Irreversible sorption
  Incorporation into humic matters

Mass transfer limitations
  Oxygen diffusion and solubility
  Diffusion of nutrients
  Solubility/miscibility in/with water

---

energy yields which thus provide less energetic incentive for microorganism degradation.

The outcome of each degradation process depends on microbial (biomass concentration, population diversity, enzyme activities), substrate (physico-chemical characteristics, molecular structure, and concentration), and a range of environmental factors (pH, temperature, moisture content, Eh, availability of electron acceptors and carbon and energy sources) (Table 1). These parameters affect the acclimation period of the microbes to the substrate. The molecular structure and contaminant concentration have been shown to strongly affect the feasibility of bioremediation and the type of microbial transformation occurring, and whether the compound will serve as a primary, secondary or co-metabolic substrate.

3.2. Bioavailability

The rate at which microbial cells can convert contaminants during bioremediation depends on the rate of contaminant uptake and metabolism and the rate of transfer to the cell (mass transfer). Increased microbial conversion capacities do not lead to higher biotransformation rates when mass transfer is a limiting factor (Boopathy and Manning, 1998). This appears to be the case in most contaminated soils and sediments. For example, the contaminating explosives in soil did not undergo biodegradation process even after 50 years. Treatments involving rigorous mixing of the soil and breaking up of the larger soil particles stimulated biodegradation drastically (Manning et al., 1995). The bioavailability of a contaminant is controlled by a number of physico-chemical processes such as sorption and desorption, diffusion, and dissolution. A reduced bioavailability of contaminants in soil is caused by the slow mass transfer to the degrading microbes. Contaminants become unavailable when the rate of mass transfer is 0. The decrease of the bioavailability in the course of time is often referred to as aging or weathering. It may result from:

1. chemical oxidation reactions incorporating contaminants into natural organic matter,
2. slow diffusion into very small pores and absorption into organic matter, and
3. the formation of semi-rigid films around non-aqueous-phase liquids (NAPL) with a high resistance toward NAPL-water mass transfer.

These bioavailability problems can be overcome by the use of food-grade surfactants (Boopathy and Manning, 1999), which increase the avilability of contaminants for microbial degradation.

3.3. Bioactivity and biochemistry

The term bioactivity is used to indicate the operating state of microbiological processes. Improving bioactivity implies that system conditions are adjusted to optimize biodegradation (Blackburn and Hafker, 1993). For example, if the use of bioremediation requires meeting a certain minimum rate, adjustment of conditions to improve biodegradation activity becomes important and a bioremediation configuration that makes this control possible has an advantage over one that does not.

In nature, the ability of organisms to transfer contaminants to both simpler and more complex molecules is very diverse. In light of our current limited ability to measure and control biochemical pathways in complex environments, favorable or unfavorable biochemical conversions are evaluated in terms of whether individual or groups of parent compounds are removed, whether increased toxicity is a result of the bioremediation process, and sometimes whether the elements in the parent compound are converted to measurable metabolites. These biochemical activities can be controlled in an in situ operation when one can control and optimize the conditions to achieve a desirable result.

4. Non-technical criteria

In addition to technical obstacles to bioremediation, some of the non-technical criteria that affect bioremediation are ability to achieve the required clean-up target, acceptable cost relative to other remediation options, acceptable risks in residual contaminants remaining after bioremediation, favorable public perception, favorable regulatory perception, ability to meet time limitations, and the ability to conform to space limitations.

4.1. Non-scientific factors affecting bioremediation

Several non-scientific factors hinder the development of bioremediation technologies and some of them are discussed below:

4.1.1. Regulatory factors

Regulations both drive and constrain the use of bioremediation. Regulation creates the bioremediation market by dictating what must be cleaned up, how clean it must be and which clean-up methods may be used (Caplan, 1993). The use of genetically engineered microorganisms (GEMs) presents additional regulatory hurdles. There is much debate over whether to use natural or GEMs in bioremediation. The advantages of naturally-occurring microbes currently outweigh those of GEMs.

Regulation can have an impact on bioremediation in three different ways:

1. *Creating markets*: Federal environmental programs require treatment of recurring wastes and remediation of existing wastes contaminating soils and groundwater (Day, 1993).
2. *Controlling the product*: Environmental laws and regulation may specify health and safety criteria for products before they can be marketed in USA.
3. *Toxic substances control act* (*TSCA*) *inventory*: All new chemicals marketed in US must be listed in this inventory. Naturally-occurring microbes are already considered on the TSCA inventory. There are no organism-specific TSCA regulations on naturally-occurring microbes; but, regulations specific for GEMs are under development.
4. *Other regulatory programs*: The Food and Drug Administration and the US Department of Agriculture control the introduction of human-food and soil pathogens. The EPA regulates the use of microbes as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (Day, 1993).
5. *Controlling the process*: Environmental Laws and regulations may specify how a product or equipment can be used to accomplish specific waste-management objectives. Some of the major US environmental laws which control the bioremediation process are listed below:

- Toxic Substances Control Act of 1976.
- Federal Water Pollution Control Act of 1972 amended by the Clean Water Act of 1977 and the Water Quality Act of 1987.
- Solid Waste Disposal Act of 1965 amended by the resource conservation and Recovery Act of 1976 and the Hazardous and Solid Waste Amendments of 1984.
- Comprehensive Environmental Response, Compensation and Liability Act of 1980 amended by the Superfund Amendments and Reauthorization Act of 1986.
- Clean Air Act of 1970 amended by CAA Amendments of 1977 and of 1990.
- National Environmental Policy Act of 1969.

4.1.2. Research and technical factors

Although there are a number of contaminants that are biodegradable, including petroleum hydrocarbons, alcohols and solvents, many widely used industrial chemicals such as PCBs, pesticides, coal tars, chlorinated solvents, and polynuclear aromatic hydrocarbons are not degraded so readily. So more intensive research is needed, but funding for this kind of basic research is diminishing.

Unlike the conventional treatment technologies, bioremediation technique must be tailored specifically to each polluted site. Each waste site has unique characteristics, and thus requires individual attention. As yet, official criteria for evaluating the success or failure of a particular strategy have not been established.

4.1.3. Human resource factor

Because bioremediation is a new technology, there is a lack of trained human resources in this field. A successful bioremediation program requires a multidisciplinary approach, integrating fields such as microbiology, engineering, geology, hydrogeology, soil science and project management. Universities do not offer qualifications in bioremediation engineering and such combined expertize can be acquired only through experience and training on the job.

4.1.4. Economic and liability factor

Unlike other industries, bioremediation does not result in the production of high value-added products. Thus, venture capital has been slow to invest in the technology and, as a consequence, commercial activity in R and D has lagged far behind other industrial sectors. As bioremediation is considered innovative technology, clients and regulatory agencies often scrutinize bioremediation more closely than conventional technologies. Consequently, tighter restrictions and performance standards are frequently imposed on bioremediation than on other remediation technologies. This can ultimately lead to a greater risk from a liability

standpoint if the bioremediation program does not accomplish the predetermined goals.

5. Conclusions

Each of the factors discussed above may limit the use of bioremediation in specific circumstances. All the factors are positive in some cases where bioremediation technology has been successfully completed. Knowledge of the susceptibility to biodegradation of some contaminants is still lacking and toxicity testing is becoming more important. Many reports indicate that bioremediation of petroleum hydrocarbons can lead to reduced toxicity and have been taken as evidence of favorable biochemistry in these cases.

There are many factors that limit bioavailability and have the impact of slowing the transport of specific compounds into aqueous phase where biological uptake occurs readily. The importance of bioavailability is strongly dependent on the nature of the contaminant, the soil chemistry, and the matrix. In some cases, bioavailability is relatively unimportant, while in others it may be critical. The influence of site-specific bioavailability on bioremediation must be considered.

Bioactivity includes consideration of those parameters that have long been recognized as influencing the rate of bioremediation. With current bioremediation configurations, only certain parameters can be manipulated. This suggests that certain sites may be particularly favorable for in situ strategies, because the bioactivity may be easily maintained.

US environmental regulations are complex; the rule promulgation process can often be slow. Intense congressional and public involvement may hinder the writing of regulations which reflect in the field experiences. Rapidly emerging technologies, such as bioremediation, have been delayed by governmental policies that support only proven technologies. The trend is slowly changing and for bioremediation using both indigenous and non-indigenous, naturally occurring microorganisms, the regulatory hurdles are decreasing.

Even with the obstacles discussed above, there are tremendous market opportunities for bioremediation. With the next 10 years, soil clean-up costs alone are estimated to exceed US dollar 30 billion in Europe (Caplan, 1993). This compares with the US dollar 1 billion spent thus far. If just 5% of this soil is cleaned using bioremediation, 1.5 billion dollars could be earned through biotreatment methods.

References

Adriaens, P., Hickey, W.J., 1993. In: Stone, D.L. (Ed), Biotechnology for the Treatment of Hazardous Waste. Lewis Publishers, Ann Arbor, pp. 97–120.

Baker, K.H., Herson, D.S., 1994. Bioremediation. McGraw-Hill, New York, pp. 1–7.

Blackburn, J.W., Hafker, W.R., 1993. The impact of biochemistry, bioavilability, and bioactivity on the selection of bioremediation technologies. TIB Tech. 11, 328–333.

Boopathy, R., Manning, J., 1998. A laboratory study of the bioremediation of 2,4,6-trinitrotoluene-contaminated soil using aerobic anaerobic soil slurry reactor. Water Environ. Res. 70, 80–86.

Boopathy, R., Manning, J., 1999. Surfactant-enhanced bioremediation of soil contaminated with 2,4,6-trinitrotoluene in soil slurry reactors. Water Environ. Res. 71, 119–124.

Caplan, J.A., 1993. The worldwide bioremediation industry: prospects for profit. TIB Tech. 11, 320–323.

Day, S.M., 1993. US environmental regulations and policies: their impact on the commercial development of bioremediation. TIB Tech. 11, 324–328.

Manning, J., Boopathy, R., Kulpa, C.F., 1995. A laboratory study in support of the pilot demonstration of a biological soil slurry reactor. Report no. SFIM-AEC-TS-CR-94038. US Army Environmental Center, Aberdeen Proving Ground, MD.