Bioremediation of Chlorinated-Aliphatics

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ABSTRACT

Chlorinated aliphatic chemicals such as trichloroethylene (TCE) and perchloroethylene (PCE) are some of the most ubiquitous soil and groundwater contaminants in the U.S. These potentially carcinogenic industrial solvents are the most common contaminant at Resource Conservation and Recovery act sites (Hopkins, Semprini and McCarty, 1993). Due to the low biodegradability of these compounds, bioremediation was historically considered an ineffective technique. However, the past two decades have seen an increase in research focusing on the biodegradation of chlorinated aliphatics. A clearer understanding of how bacteria degrade chlorinated aliphatics is allowing researchers to propose a variety of in-situ aerobic, anaerobic and hybrid systems for bioremediation of these chemicals that are more cost-effective than the traditional physical-chemical systems.

KEYWORDS

bioremediation, cometabolism, reductive dehalogenation, trichloroethylene, chlorinated aliphatic,

INTRODUCTION

Due to their widespread use in industry, organic solvents such as perchloroethylene (PCE) and trichloroethylene (TCE) are some of the most frequently-encountered soil and groundwater contaminants in the United States. This family of chemicals is technically known as chlorinated aliphatics, meaning that they are straight or branched chain hydrocarbons with some amount of chlorine substitution. Chlorinated aliphatics are used in plastics manufacturing, dry cleaning, semiconductor manufacturing and as general industrial solvents and degreasers (Vogel et al., 1987). These chemicals find their way into the environment through improper handling and disposal practices, where they and their intermediate degradative products pose a major health concern because of their known or suspected carcinogenicity (Ma, 2003).

When chlorinated aliphatic contamination is identified, there are several options that can be taken. The first decision to be made is whether the contamination needs to be treated (eliminated from the site), contained (stopping any contaminant migration, but leaving the contamination on-site) or left to attenuate naturally (the do-nothing alternative). Because containment, and to a greater extent, treatment are expensive options, this decision must be based on a thorough alternatives analysis.

There are several options once it is decided to treat the contamination. Treatment methods can be characterized as ex-situ or in-situ, physical-chemical or biological and active or passive. Traditionally, ex-situ physical-chemical methods were the most common methods chosen because they are quick and highly effective. Because chlorinated aliphatics are not easily biodegradable and often have low bioavailability, bioremediation was not seen as an effective option. However, because of the high cost associated with the ex-situ pump and treat remediation systems, much research over the last two decades has focused on improving bioremediation systems, particularly the in-situ methods (Devlin, 2003). Another factor behind the increasing popularity of bioremediation is the fact that it results in permanent destruction of contaminant molecules, as opposed to other treatment methods in which the contaminant is transferred to a different media where it must again be dealt with (National Research Council, 1993). The purpose

of this paper is to examine the current state of chlorinated aliphatic bioremediation by reviewing the literature pertaining to the molecular biology behind it, as well as its successes and failures in experimental applications.

FACTORS AFFECTING CONTAMINANT BIODEGRADATION

Bioremediation is the exploitation of naturally occurring biodegradative processes to clean up contamination (Maier, 2000). Accordingly, the biodegradation of the contaminant in question is of the highest importance. As outlined in the Maier text (2000), factors that affect the biodegradation of organics such as PCE and TCE include genetic potential, bioavailability, contaminant structure, toxicity and environmental variables.

Genetic potential refers to the fact that a microbial community's ability to biodegrade a certain compound is dependent on the ability of the microorganisms to produce specific enzymes (Maier, 2000). In the case of compounds that have chemical structures similar to those of the community's natural substrate, biodegradation should occur rapidly, following the normal metabolic pathway of most of the bacteria in the community. The natural substrate of microbial communities found in soil can generally be classified as plant-based matter. Soil microbes will usually have the genetic potential to degrade other classes of chemicals, such as pesticides, that they have been exposed to frequently. The genes that code for the enzymes that degrade these compounds are generally carried on plasmids, and will spread via induction as exposure to the chemical is increased. Xenobiotic compounds such as PCE and TCE have few naturally-occurring analogs and are therefore resistant to biodegradation. The importance of the enzyme methane monooxygenase in biodegradation of chlorinated aliphatics will be discussed later.

Once it is determined that a microbial community has the genetic potential to biodegrade a contaminant, the issue of bioavailability must be addressed. In order to be biodegraded the contaminant must first be taken into the cell from the environment. Ideally, the contaminant would be completely water soluble. However, as is the case with most hydrocarbons, chlorinated aliphatics have limited solubility in water (the water solubility of TCE is on the order of 1mg/L) (Atlas and Phillip, 2005). Because PCE and TCE are denser than water, they often form deposits of dense non-aqueous phase liquid (DNAPL) at the bottom of an aquifer. Both of these characteristics limit the biological uptake of the contaminant. A contaminant may also be sequestered if it is strongly adsorbed to soil solids or isolated in small pores where groundwater flow does not penetrate (National Research Council, 1993). Surfactants have been used for some time at pump and treat remediation sites to reduce sorption of organic contaminants. It has been suggested that surfactants could also be useful in bioremediation, but the potential inhibitory effects of these chemicals has slowed their adoption (National Research Council, 1993). In addition to utilization of the water-soluble portion, two other modes of microbial uptake are direct contact of cells with the organic compound and direct contact with sub-micrometer size droplets of substrate suspended in the aqueous phase. These two modes play an increasingly important role in substrate uptake as water solubility of the substrate decreases (Maier, 2000).

Chemical structure of a contaminant can have two types of effects on biodegradation: steric effects and electronic effects (Maier, 2000). Steric effects have to do with changes in the chemistry of the reaction site where degradative enzymes come into contact with the substrate. If the substrate contains functional groups or branched groups, the enzymes may be unable to recognize the substrate and degradation rates will be decreased. Similarly, the electronic characteristics of a substrate will affect its interaction with enzymes. Electronic effects deal with changes in electron density at the reaction site, which can be attributed to the propensity of the substrate's functional groups to be electron donors or electron acceptors. In general, biodegradation rates increase along with reaction site electron density.

Many contaminants exhibit an inhibitory effect on microbial metabolism when encountered at high concentrations, even if they are easily-degradable at lower concentrations (National Research

Council, 1993). Organic solvents are generally toxic by the nonspecific narcotic-type mode of action, which effectively disrupts the integrity of the cell membrane (Maier, 2000). Logically, this toxicity reduces the rate of contaminant biodegradation.

Environmental conditions also play an important role in biodegradation, largely because it is these conditions that determine the occurrence and abundance of microorganisms in the soil environment. Along these lines, the amount of organic matter in soil is a good predictor of the abundance of microbes. Because all heterotrophic microbes require organic carbon for metabolism, a soil with low levels of organic matter will not be able to support a large microbial community. In fact, areas in the lower vadose zone and the saturated zones have been found to contain numbers of bacteria up to two factors of 10 lower than soil in areas near the surface with higher organic matter contents.

Oxygen is another key environmental factor in biodegradation. Although both aerobic and anaerobic biodegradative pathways exist, the aerobic ones are generally much faster. In the case of in-situ treatment, soil porosity and water content are important factors determining the availability of oxygen for biodegradation (Philip and Atlas, 2005). Obviously, as the amount of pore space in soil decreases there is less room for oxgen in the bioremediation matrix. Also, because the solubility of oxygen in water is limited, increasing soil moisture content decreases oxygen availability.

Because hydrocarbon contaminants are composed primarily of carbon and hydrogen, degradation of these substrates can create a demand for nutrients such as nitrogen and phosphorous which are essential for bacterial growth. This situation is the bioremediation parallel of the nutrient deficiencies commonly encountered in the treatment of certain industrial wastewaters. For this reason, addition of nitrogen and phosphorous can increase biodegradation rates.

Finally, although microbial degradation has been observed in a wide range of conditions, rates are likely to be higher in environments with warmer temperatures, slightly alkaline pHs, low salinity and water activity values of 38-81% of available soil pore space (Maier, 2000).

DEGRADATION MECHANISMS OF CHLORINATED ALIPHATICS

As mentioned previously, the chemical structure of chlorinated aliphatics renders them considerably resistant to biodegradation. A clearer understanding of the biodegradation pathways for PCE and TCE can be obtained by considering some general trends in the degradation of all aliphatic compounds.

Importantly, biodegradation rates are reduced as the number of halogen substitutions in the molecule increases. This is because unlike most organic compounds that act as electron donors, the highly electronegative polyhalogenated compounds are able to act as electron acceptors when environmental conditions are favorable to reduction. Because of this characteristic, molecules with low numbers of halogen substitutions are better suited for aerobic degradation while the highly-halogenated molecules are more effectively degraded anaerobically (Eweis et al., 1998). The difference between aerobic and anaerobic degradation pathways will be explained subsequently.

A second general trend is that biodegradability is decreased with increasing chain branching. This phenomenon is due to steric effects as branching increases the likelihood of functional group interference at the enzyme reaction sites (Eweis et al., 1998).

Finally, it is interesting to note that aliphatics in the C_{10} – C_{18} range are the most easily degradable. Chains shorter than this are increasingly water soluble. As the contaminant is increasingly present in the aqueous phase, (i.e. more bioavailable) it is more likely that toxicity

characteristics will be observed, limiting degradation rates. On the other hand, longer chain molecules have low water solubility values and are not sufficiently bioavailable to be broken down quickly (Eweis et al., 1998).

As explained in the Maier text (2000), biodegradation of TCE occurs by one of three mechanisms. The first is substitution, in which halogens on a mono or di-halogenated compound are substituted by a hydroxyl group. Because this substitution only works to degrade mono and di-halogenated compounds, it is not a feasible solution for remediation of PCE or TCE contamination but could perhaps be used to degrade the intermediate degradative products of these compounds. An example of dehalogenation by substitution is shown below.

Substitution: $CH_3\text{-}CH_2Cl + H_2O \rightarrow CH_3\text{-}CH_2Cl + H^+ + OH^- \rightarrow CH_3CH_2OH + H^+ + Cl^-$

The second mechanism is oxidation, or the insertion of an oxygen atom into the compound. The oxygen is incorporated by a type of enzyme known as oyxgenase. Mono- and dioxygenase enzymes are produced by bacteria to oxidize a variety of organic substrates such as methane, ammonia and toluene. However, they do not have exact substrate specificity and therefore will degrade other organics present in the substrate, such as TCE or PCE. This coincidental degradation is known as cometabolism. Several oxygenase-substrate cometabolism systems have been observed to degrade chlorinated hydrocarbons, including methane monooxygenase produced by methanotrophs, toluene dioxygenase produced by toluene-degraders and ammonia monooxygenase produced by *Nitrosomonas* bacteria (Maier, 2000). Obviously, cometabolic oxidation processes can only occur in aerobic environments. Shown below are the oxidation reactions performed by methanotrophs when degrading methane (1) and cometabolizing TCE (2 and 3).

Oxidation (2): $CH_4 + O_2 \xrightarrow{\text{methane monooxygenase}} CH_3OH \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2 + H_2O$

Oxidation (2): $ClHC{=}CCl_2 + O_2 \xrightarrow{\text{methane monooxygenase}} ClHCOCCl_2 \rightarrow ClHC(OH)\text{–}(OH)CCl_2 \rightarrow HCOOH + CO + 3Cl^- + 3H^+$

Oxidation (3): $ClHC{=}CCl_2 + O_2 \xrightarrow{\text{methane monooxygenase}} ClHCOCCl_2 \rightarrow ClHC(OH)\text{–}(OH)CCl_2 \rightarrow CHO\text{–}COOH + 3Cl^- + 3H^+$

The final mechanism occurs under anaerobic reducing conditions, and is known as reductive dehalogenation. This process, mediated by reduced transition metal complexes, sequentially reduces PCE to TCE to dichloroethylenes (cis- and trans-DCE), vinyl chloride (VC) and ethene (Kao et al., 2003). In the first step of the process electrons are transferred from the reduced metal to the chlorinated organic molecule, which produces an alkyl radical and a free halogen. The alkyl radical can then pick up a hydrogen atom or lose a second halogen to form an alkene (Maier, 2000). Shown below are the reactions involved in reductive dehalogenation of perchloroethylene to trichloroethylene and trans-dichloroethylene.

Reductive Dehalogenation (1): $Cl_2HC\text{–}HCl_2 + H^+ + e^- \rightarrow Cl_2HC\text{–}CH_2Cl$

Reductive Dehalogenation (2): $Cl_2HC\text{–}HCl_2 \rightarrow ClHC{=}ClH + Cl^-$

As was mentioned previously, highly-halogenated aliphatics are better-suited to degradation through reductive dehalogenation. However, this process has been noted to result in incomplete degradation and accumulation of intermediate products such as VC that are in fact more toxic than PCE or TCE. These smaller toxic intermediates would be more efficiently degraded aerobically. For this reason several researchers have proposed treatment schemes for chlorinated aliphatics involving an initial anaerobic period to break the large molecules down into smaller ones, followed by an aerobic period that would fully degrade the smaller intermediates (Devlin, 2003).

BIOREMEDIATION TECHNIQUES

SITE CHARACTERISTICS

Because of the innumerable different techniques and treatment configurations that have been proposed for bioremediation projects, there is no one set of ideal site characteristics. However, it is possible to make some generalizations about the site characteristics that are favorable for in-situ bioremediation. The National Research Council's 1993 book *In Situ Bioremediation: When does it work?* lays out the following lists of desirable site characteristics. For instances of engineered bioremediation:

- High hydraulic conductivity for water-circulating systems
- High permeability for air-circulating systems
- Relatively consistent subsurface geology
- Low concentration of residual contaminants

For instances of intrinsic bioremediation, in which engineered systems are not used:

- Low variability in groundwater flow conditions
- Presence of natural pH buffers (i.e. carbonate hardness)
- High levels of the electron acceptors that play a key role in anaerobic dehalogenation
- Presence of nitrogen and phosphorous

AEROBIC METHODS

As explained above, aerobic degradation of chlorinated aliphatics occurs through heterotrophic cometabolism. For cometabolism to effectively degrade a contaminant, the ratio of normal substrate (methane, toluene, etc.) to contaminant must normally be quite large. For this reason, many aerobic bioremediation schemes are based on injection of supplemental organic substrate into the contaminated area. This injection of organic substrate – as well as injection of nutrients such as Nitrogen and Phosphorous – in order to stimulate growth of soil bacteria and cometabolism is known as biostimulation.

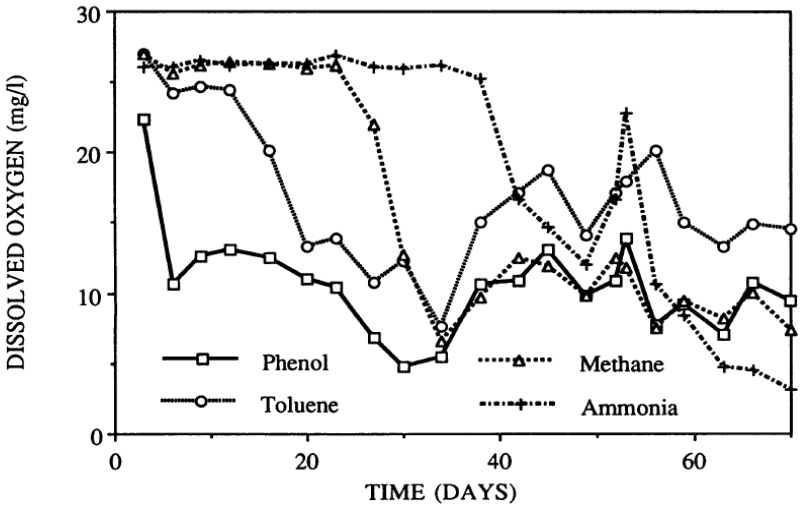

Figure 1 - Taken from McCarty et al., 1993

The phenomenon of biostimulation was discovered using natural gas as a substrate; accordingly some of the first studies done used methane. According to an early study done by McCarty, el al. (1991), degradation of 1375kg of TCE from a 480,000 cubic meter aquifer containing a contaminant load of 1617kg TCE would require 5200kg of methane and 19200kg of oxygen. Field and microcosm studies by Hopkins, Semprini and McCarty (1993) showed that phenol, toluene and ammonia could also serve as biostimulation substrates. Figure 1 above shows the oxygen uptake of several test columns of TCE-contaminated soil to which the various substrates were added. Results of this study showed that phenol stimulated the largest increase in degradation of TCE, followed by toluene, methane and ammonia.

Whereas the McCarty study cited above experimented with biostimulation by injection of various carbon sources, research done at the Savannah River Site near Aiken, South Carolina, (Brockman et al., 1995) studied the effect of Nitrogen and Phosphorous injection as well. The study began with a 21-week period of air injection as a control. Following the control period a mixture of methane and air was injected for 35 weeks. During this period methanotrophic MPN counts and TCE biodegradative potential increased initially, and then declined. After relating the varying levels of methanotrophic and TCE-degrading activity to their location relative to the injection wells, this decline was attributed to nitrogen- and phosphorous-limiting conditions. During the final period of the study, nitrous oxide and triethyl phosphate were added to the air and methane mixture for 13 weeks. As a result of nitrogen and phosphorous injection, methanotrophic MPN levels in sediment samples were found to be one to three orders of magnitude higher than during the control period (see Figure 2 below) and the number of samples exhibiting TCE biodegradative potential increased by three orders of magnitude. This study clearly shows the important effect of nutrient availability on bioremediation. However, it should be remembered that nutrient addition is not always necessary. Philip and Atlas (2005) point out that in cases where background levels of Nitrogen and Phosphorous in the soil are sufficiently high, nutrient addition is not expected to increase biodegradation rates. The addition of certain forms of nutrients may, in fact, work against bioremediation by exerting a competing oxygen demand.

The Brockman study is also notable in that it used an innovative injection system. While the majority of the literature on bioremediation of chlorinated aliphatics describes injection and extraction of liquid solutions through vertical wells, this study used horizontal wells and injected gaseous substrate. As is also the case with vertical wells, a vacuum was applied to the extraction well to promote diffusion from the injection well. Because one horizontal well can create an area of influence as large as a series of vertical wells, only one injection well was required. Also,

because the gaseous substrate had better diffusivity properties and was less likely to sorb to soil solids than a liquid, the required volume for injection was reduced.

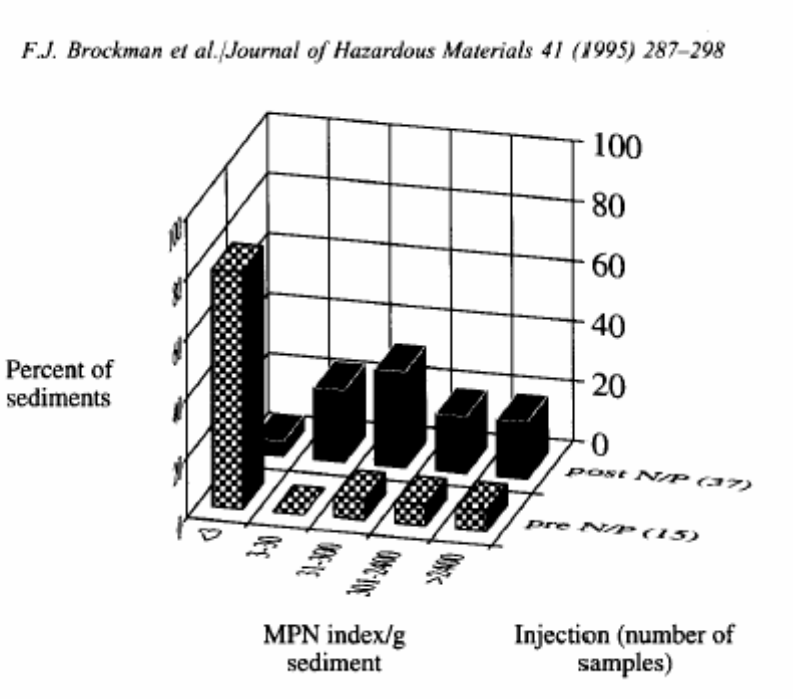

Figure 2 – Percent of sediment samples containing methanotrophs before and after nitrogen and phosphorous addition - Taken from Brockman et al., 1995

Biostimulation is not effective at sites where indigenous microbes are not genetically capable of producing the enzymes necessary for degradation of the contaminant or where the proper bacteria exist but not in sufficient numbers. In such cases some have gone one step further, attempting to increase the genetic biodegradative potential of the in-situ microbial community. This method is known as bioaugmentation and its effectiveness is the subject of much debate. According to Philip and Atlas (2005) there are three principle types of bioaugmentation. The first is the addition of specific bacterial strains that are known degraders of the target contaminant – known as allochthonous microorganisms – to the site. The second is the reinjection of bacteria that have been extracted and exposed to increasing concentrations of the contaminant in the lab. Theoretically, this practice selects for those bacteria with the desired degradative potential. The third type of bioaugmentation involves the addition of bacterial inocula derived from domestic sewage sludge or composting operations. These consortia may have no more degradative ability than the indigenous microorganisms, but because they are easy to obtain they are often marketed fraudulently to engineers as a cheap and quick solution.

The debate over whether or not bioaugmentation is an effective technique hinges on several ideas (Vogel, 1996). The first is that indigenous organisms are often more adaptable than is thought, as well as better distributed throughout the soil and closer to areas of historic contamination. However, proponents of bioaugmentation arguer that the dispersed distribution of indigenous microorganisms means that strategically added microorganisms may be in a better position to degrade recent spills. Secondly, some believe that allochthonous bacteria can be more resistant to toxicity than indigenous bacteria. While this may be true, the benefits of a toxic-resistant strain may be outweighed by the potentially poor performance of that strain in the particular environmental and ecological conditions of the site. Indigenous microorganisms have adapted to survive under a certain set of temperature, pH and moisture conditions as well as to

compete with other indigenous soil microbes. Added bacteria may not have these advantages. Finally, it has been reported that migration of bacteria added through injection wells is minimal. This can result in localized accumulations of bacteria that clog the well head.

At sites where pollution persists primarily as DNAPL, complete remediation is oftentimes not feasible. Instead, efforts are directed at preventing further migration of the pollutant plume and additional contamination. One interesting and cost-effective new approach is the installation of permeable reactive zones surrounding the plume (Kao et al., 2004). The reactive zones are constructed by trenching a volume perpendicular to the direction of subsurface flow and backfilling with an organic substrate. As contaminated groundwater flows through these zones, also known as biobarriers, the added substrate encourages bacterial growth, contaminants are biodegraded through cometabolism and clean groundwater emerges. Kao's group demonstrated that biological sludge cake from domestic wastewater treatment and sugar cane molasses can serve as effective media for these so-called 'biobarriers'. Advantages of this type of media are its high carbon content, high bioavailability and low cost.

ANAEROBIC METHODS

Most soil environments lack sufficient oxygen to sustain significant methanotrophic populations and, therefore, natural attenuation of chlorinated aliphatic contamination most often occurs by anaerobic dehalogenation. Some research directed at increasing the speed of this process has focused on providing supplemental electron donors to stimulate the dehalogenation reaction. A study performed by Ma et al. (2003) demonstrated the effects of providing the anaerobic soil environment with such a source of electron donors. In this study hydrogen gas was transferred to PCE-contaminated soil through polyethylene hollow-fiber membranes similar to those used in water filtration. Although much of the hydrogen was consumed by methanogens in competition with the dehalorespirers, nearly all of the PCE was reduced to ethene. These results are notable not only because of the biostimulation effect that hydrogen gas had on the dechlorinating bacteria, but also in that the PCE was degraded completely, leaving low levels of toxic intermediates. The study also suggested that addition of fermentable organic material may be an efficient method of supplying electron donors.

Further study of the addition of fermentable organics with the goal of stimulating anaerobic dehalogenation has occurred at the Avco Lycoming Superfund site in Williamsport, Pennsylvania (U.S. E.P.A., 2000). At this site pilot studies indicated that addition of molasses to TCE-contaminated groundwater would successfully stimulate microbial activity, increasing oxygen uptake and making redox conditions increasingly favorable for anaerobic dehalogenation. Full-scale injection of molasses into groundwater takes place in 20 four inch wells throughout the site. At the end of the initial 18-month study period levels of TCE, DCE and VC had been reduced by 90%.

HYBRID SYSTEMS

An interesting example of a hybrid aerobic-anaerobic system was tested by Devlin, Katic and Barker (2004). The testing was performed at an aquifer contaminated with PCE and other chlorinated organics, and combined an anaerobic nutrient injection well (NIW) and aerobic biosparging. The study was configured as a three-sided alley, with sheet metal walls extending down to an aquitard confining the groundwater flow. The water first passed by the NIW, which injected pulses of benzoate substrate to enhance reductive dechlorination, degrading the PCE molecules into smaller intermediates. The water then passes through an aerobic biosparging zone, which stimulates cometabolism of the smaller intermediate molecules. The study concluded that sequenced bioremediation is an advantageous technique, and recommends that further study be done in this area.

ENZYME PREPARATIONS

One potential treatment technology described by Alexander (1994) is the mass-production of enzymes such as peroxidase or methane monooxygenase. The enzymes could be made into stable preparations of soluble or immobilized form and kept on site at facilities that handle

chlorinated aliphatics. Production of enzyme preparations would probably be cost-prohibitive for large bioremediation projects, but such preparations could possibly be used in emergency situations. It is striking that Alexander says nothing about the problems that could be anticipated with respect to contact between the applied enzymes and the contamination. For this reason it is doubted that enzyme preparations will prove to be an efficient bioremediation technology.

CONCLUSION

Chlorinated aliphatic pollution of soil and groundwater is an important problem facing environmental engineers today. There is a need for cost-effective technologies for remediation of these chemicals, which is why much attention has been paid to in-situ bioremediation. A large body of research performed over the last two decades shows that bioremediation is an efficient way of degrading chlorinated aliphatics. An additional benefit of bioremediation is that it results in destruction of the contaminant, instead of transfer of the contaminant to a media that is thought to be less dangerous. As more scientists and engineers become familiar with the different bioremediation processes reviewed in this paper, rapid advances in the implementation of this technology can be expected.

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