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Engineering concepts for *in situ* bioremediation*

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Abstract

Most organic materials that contaminate soil and the subsurface environment are readily degraded by natural biological processes. Thus, bioremediation can be thought of as a highly successful purification process. However, some organic molecules are naturally refractory to biodegradation, the proper microbial population is not present, or environmental conditions are not suitable to biodegradation so that the compounds become recalcitrant to biodegradation and persist in the environment for years. Where natural processes are slow, the best strategy may still be to wait for the natural degradation to occur because of the current high costs and lack of reliability of engineered solutions. In other cases, the natural recalcitrance may be changed by introduction of degrading populations of microorganisms or by changing the environmental conditions by introduction of chemicals or through mixing and dilution. The potential success of engineering procedures to enhance degradation rates also depends to a large degree upon the complexity of the hydrogeology at a given site. With complex hydrogeology, most remediation approaches are rendered difficult if not impossible. This must be recognized by regulatory authorities and the public. In such cases alternative strategies to site remediation itself should be sought. More attention to such alternatives is needed in order to reduce unproductive expenditure of scarce resources.

Introduction

A question often asked is: "Do biological approaches have promise for cleanup of contaminated soils and groundwater?" The answer is a clear and unambiguous "yes". If it were not for the success of natural biological processes, the earth would be very much unlike it is today. The question is not whether *in situ* biological degradation is successful, but how to better exploit this natural process in the few cases where the natural process is too slow to meet our needs or desires.

Engineered biological treatment processes have been used for well over a century for degradation of organic chemicals that are unwanted and may otherwise pollute the environment. Included are municipal and industrial waste-

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water treatment plants, aerobic and anaerobic sludge treatment facilities, soil farming, oxidation ponds, and sanitary landfills. These facilities have been used successfully for degradation of a broad range of natural and man-made (xenobiotic) organic chemicals. In general, these engineered processes take advantage of the concept of "microbial infallibility." This concept suggests that organic compounds that are produced biologically, can be destroyed biologically.

While it may have been thought that the concept of microbial infallibility applied to all xenobiotic organic chemicals as well, this was brought into question in the early 1950's with the introduction of synthetic detergents and pesticides. Both began to have harmful effects on the environment, and it was soon realized that a part of the problem was their resistance to degradation by microorganisms. While such recalcitrance is often thought to be a problem only with some xenobiotics, it is associated with many natural organic materials as well [1].

The relatively recent awareness of widespread surface and subsurface soil and groundwater contamination with hazardous chemicals has brought new engineering challenges. The scientific and engineering principles that have been developed for design and operation of biological treatment processes are generally applicable for many organic contaminants that are readily degraded by microorganisms. However, there are some important differences that make the new contaminated environments much more difficult for engineering solution. Included are the complex nature of the subsurface systems and the lack of adequate means for their characterization. Also of significance is the lack of control one has over such natural environments compared with engineered reactors. These differences greatly increase the cost and uncertainty of success with any proposed solution. Biological approaches are receiving increased emphasis for remediation because they offer promise for ridding the environment of harmful contaminants, rather than simply moving them from one location to another. Some of the scientific and engineering concepts that are useful for site remediation by biological processes and their limitations are addressed in the following.

Factors affecting compound recalcitrance

In order to engineer a system for *in situ* bioremediation, the first step is to identify the factors causing the organic contaminants in question to resist natural biodegradation. Alexander [1] listed six factors that bring about molecular recalcitrance:

(1) A structural characteristic of the molecule prevents an enzyme from acting.
(2) The compound is inaccessible.
(3) Some factor essential for growth is absent.
(4) The environment is toxic.

(5) Requisite enzymes are inactivated.
(6) The community of microorganisms present is unable to metabolize the compound because of some physiological inadequacy.

The first factor suggests that for biodegradation to occur, the chemical must be inherently biodegradable. The next four factors are related to environmental conditions that are necessary for biodegradation to take place. The last indicates that appropriate organisms with capability for degrading the chemical must be present. If biodegradation does not occur naturally, then at least one of the factors listed is likely to be operative. In order to bring about biodegradation, the factors imparting recalcitrance must be corrected. If none of the six factors are effective, then natural purification processes will most likely occur, resulting in biodegradation of the compounds of concern.

The objective of engineering strategies for enhancing *in situ* biodegradation is to correct factors effecting recalcitrance. The three general areas of molecular structure, microorganism presence, and environment are discussed in the following.

Molecular structure

Most natural and xenobiotic compounds are biodegradable by microorganisms through normal functions of metabolism for energy and growth. Here, a portion of the organic material, serving as a primary energy source, is converted to end products through oxidation/reduction processes, and a portion of the organic carbon is synthesized into cellular material. Such conversions can take place in aerobic environments in which oxygen serves as the terminal electron acceptor, or in anaerobic processes where nitrates, sulfates, carbon dioxide, or the organic compounds themselves may serve as electron acceptors. Where bacteria use the compounds of concern for a primary energy source, the pathways, end products, and rates of reaction are often sufficiently well known so that engineered systems can be designed and operated to bring about the particular conversions desired.

Aromatic hydrocarbons and chlorinated aliphatic compounds are among the most commonly found groundwater contaminants. Many of the former are the more soluble components in gasoline (benzene, ethylbenzene, toluene, xylene) and result because of hydrocarbon spills or leakage. The latter result from disposal of chlorinated solvents which have commonly been used throughout industry for degreasing, and include tetrachloroethylene (PCE), trichloroethylene (TCE), and 1,1,1-trichloroethane (TCA).

The aromatic hydrocarbons can generally be used readily as primary substrates under aerobic conditions [2,3], but in the absence of oxygen, degradation rates are slow. Formerly, these compounds were believed not to be degradable anaerobically. However, in recent years, much evidence has been obtained to indicate this is not the case [4–10], and anaerobic degradation takes place

naturally in the environment. However, good understanding of the microorganisms involved and their prevalence, the anaerobic pathways of degradation, environmental requirements for the degrading microorganisms, and reaction rates is very limited. Research in this area is difficult because of the slow growth rates of the degrading microorganisms. However, this area is most promising because anaerobic environments normally exist when contamination is high. If procedures can be found to confirm that natural degradation is occurring at a given site at reasonable rates, then scarce resources may not need to be expended for remediation at such a site. In addition, with a better understanding of the factors involved, engineering methods for enhancing anaerobic degradation might be developed.

The chlorinated solvents and many other halogenated aliphatic compounds were previously thought to be recalcitrant both aerobically and anaerobically. Even now, no microorganism is known that can use either PCE, TCE, or TCA as a primary organic source for energy or growth. However, it is still possible to biologically degrade such compounds through the process of cometabolism [11,12]. Cometabolism is the biotransformation of a compound by a microorganism that gains little energy or organic carbon for growth from the compound. It is a fortuitous transformation brought about by enzymes or cofactors produced by the organism for other purposes. Examples are oxidation by cometabolism of chlorinated aliphatic compounds such as trichloroethylene, 1,2-dichloroethylene, and vinyl chloride by microorganisms that produce non-specific oxygenases. One of the most studied groups of cometabolizing microorganisms are methanotrophs, which in the presence of oxygen use methane as a primary energy and carbon source [13–19]. Other cometabolizing microorganisms are toluene oxidizers, nitrifying bacteria, and ethane, propane, and ethylene oxidizers [20–24]. The oxygenases produced by these microorganisms can initiate oxidations of a broad range of aromatic and aliphatic compounds under aerobic conditions.

Many of the halogenated aliphatic compounds can also be reduced by cometabolism [25–32] . Such reduction often involves the removal of a chlorine atom from the molecule and replacement with hydrogen [33]. In this way, TCE can be transformed into 1,2-dichloroethylene, and then into chloroethlyene (vinyl chloride). Vinyl chloride can even be transformed into ethylene by this process. However, as chlorine atoms are removed, the process becomes slower. Such anaerobic reductions occur frequently in the subsurface environment such that many degradation products in addition to the original contaminating compound are often found at contaminated sites. Figure 1 is a composite of many of the known pathways for chemical and anaerobic biological transformations of halogenated compounds taken after [34].

It is now commonly proposed that highly chlorinated compounds such as PCE, carbon tetrachloride, and the more highly chlorinated congeners of PCB be reduced to less chlorinated compounds using anaerobic conditions where

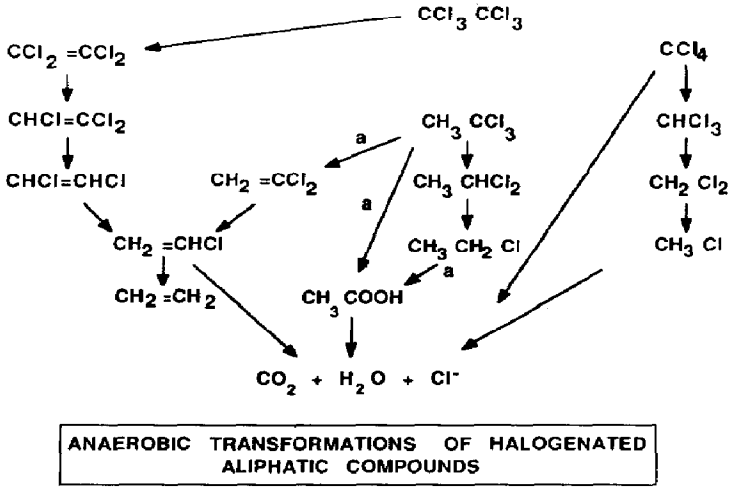

Fig. 1. Chemical and biological transformation pathways of selected chlorinated aliphatic compounds under anaerobic conditions. Arrows with 'a' indicate chemical transformations; other arrows represent biological transformation (after [34]).

rates of reduction tend to be faster than aerobic oxidations. Then aerobic processes can be used to complete the degradation process since they tend to be faster for the less chlorinated compounds. The overall degradation of compounds in this way may partially rely on cometabolic processes to initiate reactions, forming other compounds which may be used as primary carbon sources for the same of other organisms. Often with cometabolism, communities of organisms participate in the complete mineralization of an organic compound. Attempts are now being made to take advantage of cometabolism for degradation of halogenated aliphatic compounds at contaminated sites [19,35]. Since experience with engineered cometabolic processes is almost nonexistent at present, degradation through cometabolism must be considered as an innovative approach to bioremediation.

Environmental factors

Figure 2 illustrates contamination of soil and groundwater by leakage from a storage tank, a common way by which contamination with liquids occurs [36]. As the liquid is pulled downward by gravity, residuals left behind contaminate the surface soil, the unsaturated (vadose) zone, and finally the aquifer containing the groundwater itself. After the leakage is found and stopped, and the most highly contaminated soil around the tank is excavated, one must then deal with a lower-concentration residual in the soil, the vadose zone, and the groundwater. If the contaminating liquid is a mixture of many different compounds, then each may move and be transformed at different rates. Biological and chemical transformations may not lead to mineralization, but may

result in producing other organic chemicals that may be either less or more harmful than the original. Organics may become strongly sorbed onto subsurface minerals or may penetrate into cracks so that they are not accessible by microorganisms or their enzymes.

The relatively homogeneous subsurface environment indicated in Figure 2 is ideal, but seldom encountered. In such a case, groundwater flow direction and rate might be determined from relatively few observations of piezometric heads and data from pumping tests. Subsurface environments often are much more complex than this, some perhaps as illustrated in Fig. 3 [36]. Layering

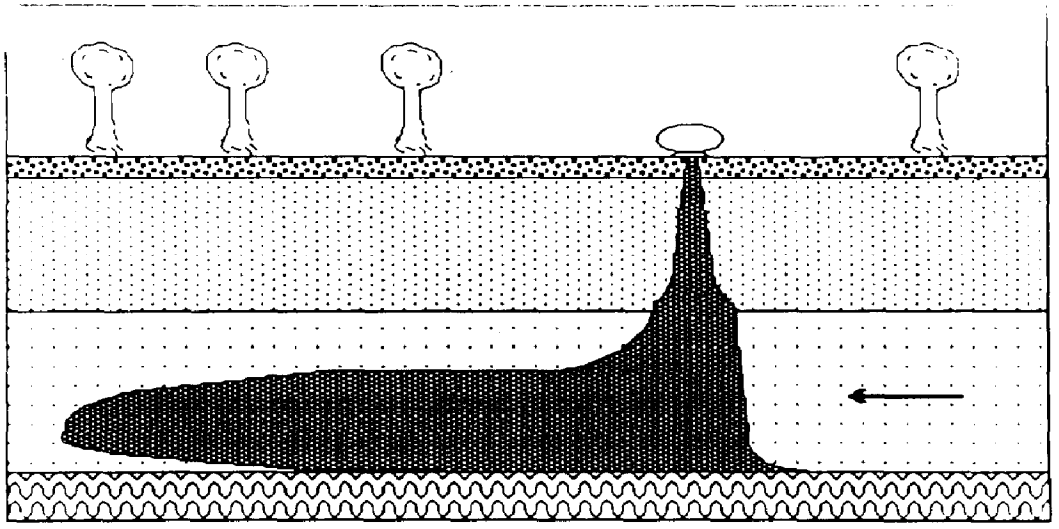

Fig. 2. Contamination of a homogeneous subsurface system.

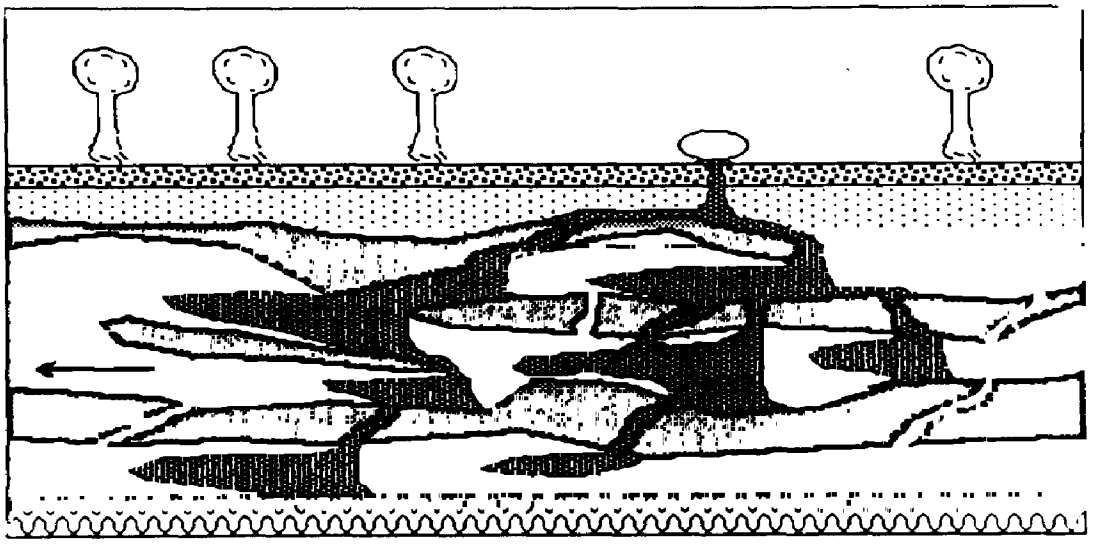

Fig. 3. Contamination of a heterogeneous subsurface system.

of permeable (sands and gravels) and less-permeable (silts, clays, rock) strata are common and may contain discontinuities that could result from faults or large-scale stratigraphic features. Conductivity of water and contaminants through rocks and other such barriers may result from joints and fractures that are difficult to locate and to describe. The mixture of gravel, sand, silt, clay, and organic matter of which the subsurface environment consists can vary widely from location to location, as can the grain-size distribution and mineral composition within each broad class of subsurface strata. In addition, abandoned wells can often provide passage ways between separated aquifers. Recalcitrance of contaminants in such systems may result from high concentrations which are toxic, from presence of the organics in fissures, strong sorption to particle surfaces or diffusion into small pore spaces in minerals, rendering the contaminants inaccessible to microorganisms and their enzymes. Sorbed compounds often desorb slowly, and this often becomes the limiting factor affecting rates of biodegradation as well as removal by pump-and-treat methods [36]. Recalcitrance may also result from insufficient required nutrients, such as nitrogen and phosphorus for bacterial growth [2, 3]. For aerobic treatment, optimal concentrations of ammonia or nitrate nitrogen are in the range of 2 to 8 pounds per 100 pounds (45.3 kg) of organic material, while inorganic phosphorus requirements are about one-fifth of this [34]. When these nutrients are below optimum levels, rates of biodegradation slow considerably and may be more dependent upon rates of nitrogen and phosphorus regeneration than on other factors. With anaerobic degradation, nutrient needs are generally less, but organism growth rates are slower. The absence of suitable electron acceptors is another factor that can affect biodegradability. For aromatic hydrocarbons, degradation rates are generally enhanced through aerobic decomposition. Thus, introduction of oxygen can be useful. Generally, the quantity of oxygen required is similar to the mass of contaminants present. In complex subsurface systems, getting the oxygen to the areas of need can prove difficult.

Frequently, when environmental conditions are not appropriate for biodegradation to occur, potential solutions often involve addition of chemicals [2, 3, 35, 19]. This is perhaps not difficult with surface contamination, but may be nearly impossible with some subsurface contamination, depending upon the hydrogeology. With the latter, conditions that make pump-and-treat difficult, also render efforts at bioremediation difficult. If it is difficult to pump contaminants out of the ground, then it is also difficult to pump chemicals or microorganisms into the ground to reach the contaminants. In such cases, biological approaches may not offer significant time advantages over pump-and-treat. The main advantage of bioremediation is likely to be an environmental one, the contaminants are destroyed with a minimum of disruption of the surface environment.

In some cases, costs may be significantly reduced as well. In some cases,

proper environmental conditions may be obtained by moving contaminants to effect dilution or mixing with natural chemicals in the subsurface system. Dilution by mixing of contaminated and uncontaminated groundwater can reduce contaminant toxicity. Also with dilution, alternate electron acceptors, such as oxygen or nitrates, or essential nutrients, such as nitrates, phosphates, and iron, that are present in uncontaminated water, may be brought together with the contaminants for better biodegradation. Again, better methodologies for predicting the outcome of this strategy are needed.

Where environmental conditions are suitable, and where the proper microbial populations are present, complete mineralization of organic contaminants can occur, even within the most complex hydrogeological environments. Even where environmental conditions are not ideal, degradation of many organic chemicals may take place at reduced rates, with half-lives on the order of one or two years. In such cases, the correct strategy may be to leave the contaminants alone, and allow the problem to be rectified by natural processes. Environmentally, this may be the best position to take. The difficulty here is in obtaining evidence that would convince ourselves, the regulatory authorities, and the public that such natural processes are indeed occurring. Also difficult is making good estimates of the time-frame for natural purification to occur. Currently, we do not know what evidence to collect to prove the occurrence of natural degradative processes, nor how to collect it. This is a most important area of need.

Microbial presence

With contaminants that are known to be readily biodegradable, the absence of a suitable microbial population may also be a factor. Methodologies for determining microorganism presence are under development. Some include the simple exposure of aseptically obtained soil to the contaminants of concern under ideal chemical conditions for biodegradation. If the microorganisms are naturally present, then degradation of the contaminant will occur. Others attempt to identify the presence of species known to biodegrade the compounds of interest, or propose the use molecular probes that can identify the presence of specific microorganisms, nucleic acid sequences, or enzymes that are key to compound degradation. These more sophisticated techniques are not yet fully developed, but may offer promise for the future.

If appropriate organisms are not present, then they may be introduced into the surface or subsurface environment [37]. Such organisms may be natural, but not ubiquitous in nature. Their growth and introduction into a new system may thus be acceptable. An important question is whether such specialized organisms can survive in the new environment, and if so, can they be transported to the place of need. If the hydrogeology is complex, then this may be most difficult. In other research, attempts are being made to engineer micro-

organisms that are capable of degrading organic compounds that are inherently recalcitrant. The potential use of such organisms raises societal concerns as well as the physical and biological barriers to successful organism introduction into the environment. Nevertheless, such approaches deserve to be explored as they will add to our overall knowledge of the biodegradation process.

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