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Engineering and hydrogeological problems associated with *in situ* treatment*

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Abstract Most organic materials that contaminate soil and the subsurface environment are readily degraded by natural biological processes. To this degree, in situ bioremediation can be thought of as a highly successful purification process. However, some organic molecules are naturally refractory to biodegradation, or other environmental factors induce molecular recalcitrance such as the absence of a proper microbial population or the presence of unsuitable environmental conditions. Examples of recalcitrant groundwater contaminants are soluble components of petroleum hydrocarbons (BTEX) and chlorinated aliphatic hydrocarbons (CAHs). Organic recalcitrance may be changed through introduction of degrading populations of microorganisms or by changing the environmental conditions through introduction of nutrients or other chemicals. The most significant engineering deficiency in *in situ* bioremediation is the absence of proven methods to introduce such materials into the subsurface environment for efficient mixing with microorganisms and the contaminants of concern.

Problèmes de génie et d'hydrogéologie associés au traitement *in situ*

Résumé La plupart des substances organiques qui contaminent les sols et l'environnement souterrain sont facilement décomposées par des processus biologiques naturels. A ce niveau, la biorestauration *in situ* peut être considérée comme un procédé très efficace. Cependant, certaines molécules organiques sont naturellement réfractaires à la biodégradation et d'autres facteurs environnementaux, tels que l'absence de populations microbiennes appropriées ou la présence de conditions environnementales défavorables conduisent à leur persistance. Les composantes solubles des hydrocarbures pétroliers (BTEX) et les hydrocarbures aliphatiques chlorés (HAC) sont des exemples de contaminants réfractaires dans les eaux souterraines. La résistance organique peut être modifiée grâce à l'introduction de populations de microorganismes capables d'effectuer la biodégradation ou en modifiant les conditions environnementales par l'introduction de substances nutritives ou autres produits chimiques. La lacune la plus importante au niveau du génie de la biorestauration *in situ* est l'absence de méthodes adéquates pour l'introduction de ces matériaux dans l'environnement souterrain et le mélange efficace des microorganismes et des contaminants visés.

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INTRODUCTION

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 water treatment plants, aerobic and anaerobic sludge treatment facilities, soil farming, oxidation ponds, and sanitary landfills. Such 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, which generally are produced biologically, can also be destroyed biologically.

In the early 1950s, with the increasing usage of xenobiotic organic chemicals, the concept of microbial infallibility was brought into question. The first sign of difficulty came from excessive foaming at biological treatment plants, and in streams and groundwater, following the introduction of synthetic detergents. Shortly after this, the problems with persistence of pesticides in the environment arose. It was soon realized that a major part of the problem with these chemicals 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 (Alexander, 1965).

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, and the difficulty of modifying the natural environment or introducing chemicals or other materials required for the desired biological transformations to occur. These differences greatly increase the cost and uncertainty of success of 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 this paper.

RECALCITRANCE OF ORGANIC MOLECULES

Most organic chemicals which enter the natural environment, including those

of industrial origin, are degraded biologically. In this sense, *in situ* bioremediation does occur naturally all the time. However, the troublesome chemicals that are found to persist at hazardous waste sites or in groundwaters may do so for any one or more of several factors causing molecular recalcitrance, as it was termed by Alexander (1965). In order to engineer a system for *in situ* bioremediation, the first step is to identify what factors cause the organic contaminants of concern to resist natural biodegradation, and then to modify these factors in a manner to make biodegradation possible. Alexander (1965) listed six major factors that bring about molecular recalcitrance:

(a) a structural characteristic of the molecule prevents an enzyme from acting;
(b) the compound is inaccessible;
(c) some factor essential for growth is absent;
(d) the environment is toxic;
(e) requisite enzymes are inactivated; and
(f) 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 the capability for degrading the chemical must be present. If none of the six factors is effective, then purification will most likely occur, resulting in biodegradation of the compounds of concern. When chemicals persist, then one or more of the above factors may be the cause.

The objective of engineering strategies for enhancing *in situ* biodegradation is to correct factors causing 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, sulphates, carbon dioxide, or the organic compounds themselves (through fermentation), may serve as electron acceptors. When bacteria use the compounds of concern as a primary energy source, the pathways, end products and rates of reaction are often sufficiently well known that engineered systems can be designed and

operated to bring about the particular conversions desired.

Aromatic hydrocarbons and chlorinated aliphatic hydrocarbons (CAHs) are among the most commonly found groundwater contaminants. Many of the former are the more soluble components in gasoline, which includes predominantly benzene, toluene, ethylbenzene and xylene (BTEX) 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), 1,1,1-trichloroethane (TCA) and carbon tetrachloride (CT). Several degradation products of the chlorinated solvents occur in natural systems, including other CAHs such as cis-1,2-dichloroethylene (c-DCE), trans-1,2-dichloroethylene (t-DCE), 1,1-dichloroethylene (1,1-DCE), 1,1-dichloroethane (1,1-DCA) and vinyl chloride (VC). Table 1 contains a summary of the potential for biological transformation of the BTEX and CAH group of compounds. The difference between transformation as a primary substrate and through co-metabolism is discussed below.

Primary substrates are compounds that can be used by microorganisms as electron donors for energy and growth. As such their degradation is autocatalytic in that the compounds themselves support the growth of organisms responsible for their degradation. Degradation here can be assured providing

Table 1 *Potential for BTEX and CAH biotransformation as primary substrates or through co-metabolism*

	Primary substrate		Co-metabolism		
Compound	Aerobic potential	Anaerobic potential	Aerobic potential	Anaerobic potential	Anaerobic CAH product
BTEX					
Benzene	XXX	0			
Toluene	XXX	XX			
Ethylbenzene	XXX	X			
Xylene	XXX	X			
CAHs					
CCl_4	0	0	0	XXXX	$CHCl_3$
$CHCl_3$	0	0	X	XXXX	CH_2Cl_2
CH_2Cl_2	XXX	XX	XXX		
CH_3CCl_3	0	0	X	XXXX	CH_3CHCl_2
CH_3CHCl_2	0	0	X	XX	CH_3CH_2Cl
CH_2ClCH_2Cl	Yes	0	X	XX	CH_3CH_2Cl
CH_3CH_2Cl	Yes	0	XX	a	
$CCl_2=CCl_2$	0	0	0	XXX	$CHCl=CCl_2$
$CHCl=CCl_2$	0	0	XX	XXX	CHCl=CHCl
CHCl=CHCl	0	0	XXX	XX	$CH_2=CHCl$
$CH_2=CCl_2$	0	0	X	XX	$CH_2=CHCl$
$CH_2=CHCl$	Yes	0	XXXX	X	

Notes: 0 - very small if any potential; X - some potential; XX - fair potential; XXX - good potential; XXXX - excellent potential; a: readily hydrolysed abiotically, with half life of the order of one month.

appropriate environmental conditions prevail, and necessary nutrients and electron acceptors together with at least a few of the degrading population are present. The BTEX group of aromatic hydrocarbons can generally be used readily as primary substrates under aerobic conditions (Knox *et al.*, 1985; Thomas & Ward, 1989), but in the absence of oxygen degradation may be possible but rates can be slow. Formerly, these compounds were believed not to be degradable anaerobically. However, in recent years, much evidence has been obtained to indicate that this is not the case (Reinhard *et al.*, 1984; Grbic-Galic & Vogel, 1987; Lovley & Lonergan, 1990; Major *et al.*, 1988; Vogel & Grbic-Galic, 1986; Wilson *et al.*, 1986; Zeyer & Schwarzenbach, 1986); 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. 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 microbial requirements for anaerobic degradation to occur, engineering methods for enhancing anaerobic degradation might be developed.

The chlorinated solvents and many other CAHs were previously thought to be recalcitrant both aerobically and anaerobically. Even now, no micro-organism is known that can use either PCE, TCE or TCA as a primary organic source for energy or growth. However, is it still possible to degrade such compounds biologically through the process of co-metabolism (Jensen, 1963; Leadbetter & Foster, 1959). Co-metabolism 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 co-factors produced by the organism for other purposes. Examples are oxidation by co-metabolism of chlorinated aliphatic hydrocarbons such as TCE, DCE or VC by microorganisms that produce non-specific oxygenases. One of the most studied groups of co-metabolizing microorganisms are methanotrophs, which in the presence of oxygen use methane as a primary energy and carbon source (Henson *et al.*, 1989; Janssen *et al.*, 1987; Little *et al.*, 1987; Oldenhuis *et al.*, 1989; Roberts *et al.*, 1989; Tsien *et al.*, 1989; Wilson & Wilson, 1985). Other co-metabolizing microorganisms are toluene oxidizers, nitrifying bacteria and ethane, propane, ethylene and isoprene oxidizers, (Arciero *et al.*, 1989; Ewers *et al.*, 1991; Nelson *et al.*, 1986; Nelson *et al.*, 1988; Shields *et al.*, 1989; Wackett *et al.*, 1989). 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 co-metabolism (Barrio-Lage *et al.*, 1986; Belay & Daniels, 1987; Bouwer & McCarty, 1983a; 1983b; Bouwer *et al.*, 1981; Fathepure *et al.*, 1987; Freedman & Gossett, 1989; Vogel & McCarty, 1985). Such reduction often involves the removal of a chlorine atom from the molecule and replacement

with hydrogen (Vogel *et al.*, 1987). In this way, TCE can be transformed into 1,2-DCE and VC. VC can even be transformed into ethylene by this process (DiStefano *et al.*, 1991; Freedman & Gossett, 1989). However, as chlorine atoms are removed, the process becomes slower. Such anaerobic reductions occur frequently in the subsurface environment such that in addition to the original contaminating compound many degradation products are often found at contaminated sites.

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 rates of reduction tend to be faster than in 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 co-metabolic processes to initiate reactions, forming other compounds which may be used as primary carbon sources for the same or other organisms. Often with co-metabolism, communities of organisms participate in the complete mineralization of an organic compound. Attempts are now being made to take advantage of co-metabolism for degradation of halogenated aliphatic compounds at contaminated sites (McCarty *et al.*, 1991; Roberts *et al.*, 1989). Since experience with engineered co-metabolic processes is almost nonexistent at present, degradation through co-metabolism must be considered as an innovative approach to bioremediation.

ENVIRONMENTAL FACTORS

Subsurface contamination with gasoline, other petroleum products, or chlorinated solvents often results from the inadvertent spillage of non-aqueous phase liquids (NAPLs) onto soil. As the liquid infiltrates through the soil, 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 contamination 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 production of 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 they are not accessible by microorganisms or their enzymes.

With a relatively homogeneous subsurface environment, which seldom occurs, groundwater flow direction and rate might be determined from relatively few observations of piezometric heads and data from pumping tests. However, subsurface environments often are much more complex than this.

Layered permeable strata (sands and gravels) and less-permeable strata (silts, clays, rocks) 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 passageways 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 (McCarty, 1990). Recalcitrance may also result from insufficient required nutrients, such as nitrogen and phosphorus, for bacterial growth (Knox *et al.*, 1985; Thomas & Ward, 1989). For aerobic treatment, optimal concentrations of ammonia or nitrate nitrogen are in the range of 2 to 8% by weight of organic material, while inorganic phosphorus requirements are about one-fifth of this (McCarty, 1988). 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 (Knox *et al.*, 1985; McCarty *et al.*, 1991; Roberts *et al.*, 1989; Thomas & Ward, 1989). 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 being 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, alternative 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, one does 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 limiting biodegradation. 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 of molecular probes that can identify the presence of specific microorganisms, nucleic acid sequences or enzymes that are the 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 (Omenn *et al.*, 1988). 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 microorganisms 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.

INTRODUCTION OF CHEMICALS

Perhaps the most significant challenge for *in situ* bioremediation is the introduction into the subsurface environment of nutrients, other chemicals or perhaps organisms that are needed to create a suitable environment for compound destruction and mixing the introduced materials with indigenous microorganisms and the contaminants to be degraded. Chemicals to be introduced may be nutrients, such as nitrogen and phosphorous alone, an electron acceptor such as oxygen (perhaps in the form of peroxide) or nitrates, a primary substrate such as methane for co-metabolism, acids or bases for pH control or some combination of these. The easiest to add are hydrophilic chemicals that are relatively soluble in water. Among the problem chemicals are those which react by ion exchange with clays, such as ammonium, or tend to sorb or precipitate, such as phosphate. Poorly soluble gases such as methane and oxygen are also difficult because of the high energy cost for transfer from the gas phase into water and the small concentrations that can be delivered to the subsurface. A common procedure is to mix the chemicals of interest with water at the surface and introduce the dissolved chemicals by injection of the water into the subsurface. A difficulty here is that the introduced water may push away the native groundwater containing the contaminants so that the required mixing between the contaminants and the introduced chemicals will not occur. However, if the contaminants sorb somewhat to the soils, then a benefit will be obtained as the movement of the sorbed contaminants will be retarded, permitting unretarded nutrients to mix with the contaminants. Upon desorbing into the introduced water, the contaminants and introduced chemicals are thus brought together.

Another engineering consideration, especially with co-metabolism, is the problem of excessive microbial growth near the point where the primary substrate being used for enzyme induction is introduced into the subsurface. Microorganisms will tend to grow near the point of chemical introduction where concentrations are the highest. In order to avoid potential clogging, a strategy is needed that will make growth difficult near the point of injection. The periodic introduction of inhibitory chemicals such as chlorine or ozone may be required, or a strategy such as pulsing of the primary substrate and oxygen so that they do not occur together near the injection point or are injected at different points are possibilities (Semprini & McCarty, 1991). Full-scale experience with such strategies is limited and so documented guidelines for application are not available.

One strategy is to extract groundwater, treat it if necessary at the surface, introduce desired chemicals or bacteria into the water, and reinject the amended

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