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**SYNTHETIC CHEMICALS WITH POTENTIAL FOR
NATURAL ATTENUATION
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14. ABSTRACT

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Synthetic Chemicals with Potential for Natural Attenuation

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ABSTRACT: Natural attenuation of petroleum hydrocarbons is predictable and self-sustaining because bacteria able to use the contaminants as growth substrates are widely distributed. In contrast, bacteria able to grow at the expense of chlorinated aliphatic compounds are less common and the natural attenuation of such compounds is, therefore, less predictable. The purpose of this paper is to describe examples of other synthetic organic compounds that are known to be biodegradable and have the potential for natural attenuation in the field.

Keywords: natural attenuation, biodegradation, cometabolism, chlorobenzenes, chlorophenols, polychlorinated biphenyls (PCBs), chloroaliphatics, nitroaromatics.

Introduction

Attenuation of natural organic compounds such as those present in hydrocarbon fuels is predictable because the responsible microorganisms are ubiquitous in soil and in the subsurface. Bacteria able to use hydrocarbons as their source of carbon and energy under either aerobic or anaerobic conditions have a tremendous selective advantage over other members of the microbial community. Therefore, the process can be self-sustaining and is limited only by the presence of electron acceptors or inorganic nutrients (Figure 1).

In contrast, bacteria able to grow at the expense of chlorinated aliphatic compounds are not widely distributed, and natural attenuation of such compounds is, therefore, less predictable. The use of a chlorinated compound as a terminal electron acceptor (chlororespiration or dehalorespiration) can yield energy and thus provide a selective advantage to a limited range of anaerobic bacteria (Mohn and Tiedje, 1992). Many of the transformations (often called cometabolism) of chloroaliphatic compounds such as trichloroethylene yield no advantage to the bacteria that catalyze the reactions. In fact, such transformations can select against the organism because of the wasting of energy and the production of toxic metabolites.

Between the extremes of readily degradable hydrocarbons and chlorinated aliphatic compounds that serve only as electron acceptors are many other synthetic organic compounds that can provide sources of carbon and energy for bacteria. A more limited, but important, group of organic compounds containing nitrogen, phosphorus, or sulfur can

support growth by serving as sources of nutrients. The purpose of this paper is to describe examples, but not an exhaustive list, of compounds that are known to be biodegradable and have the potential for natural attenuation in the field. Some synthetic chemicals can be expected to be readily susceptible to natural attenuation, some are degraded at a limited number of sites, and some show only a limited potential. Where possible, recent review articles rather than primary literature are cited. More detailed information on many of the compounds is available in a book that provides an excellent analysis of the potential for biodegradation (Young and Cerniglia, 1995).

The first question to be asked in consideration of the potential for natural attenuation is whether biodegradation of the chemical contaminant has been reported. The question could be phrased, "Does the biology exist?" Biodegradation of some of the compounds has been studied extensively under field conditions. Transformation of others has only recently been discovered in laboratory systems or wastestreams. Such laboratory studies should not be ignored because the processes discovered in such systems are catalyzed by bacteria obtained from the field. Laboratory studies are essential for revealing the mechanisms of the reactions and the conditions required for the process. They can also determine whether the process provides energy or nutrients and thus a selective advantage to the bacteria that catalyze the reactions.

The second question is whether the activity or the necessary specific organisms are present at the site under consideration (Figure 2). A considerable amount of effort has been wasted on enumeration and identification of bacteria at hy-

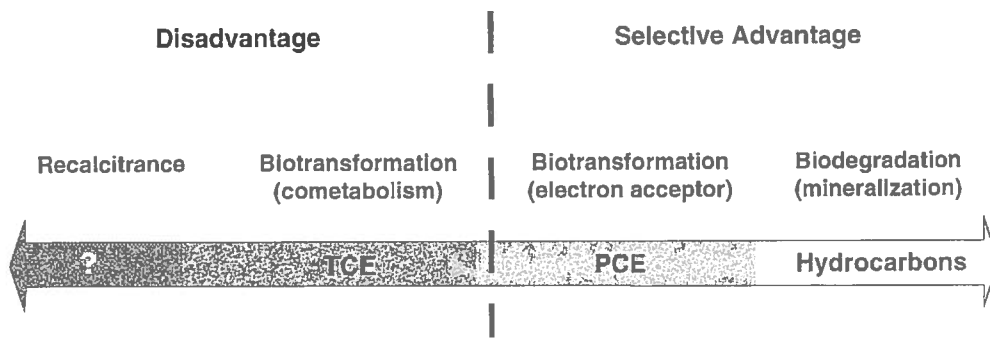


Figure 1. Contaminant-microbe interactions. When microorganisms encounter organic contaminants, several responses are possible. Both biodegradation (use of the contaminant as a source of carbon and energy) and biotransformation, involving use of the contaminant as an electron acceptor coupled to energy production and growth, provide the organism with a selective advantage. Such processes can be self-sustaining and very useful for natural attenuation. In contrast, biotransformations that do not provide energy or nutrients are much more difficult to sustain in the environment because they divert energy from the microorganisms.



Figure 2. Distribution of specific degraders. Bacteria able to degrade the light hydrocarbons in petroleum distillates are ubiquitous. The ability to degrade many synthetic chemicals is widely distributed, but patchy and difficult to predict in the soil and subsurface. Microorganisms able to degrade other synthetic compounds are found only rarely and only in specialized ecosystems including waste treatment systems and contaminated sites. Detection of such strains at a contaminated site is evidence that natural attenuation is taking place. The number of simple, common organic compounds thought to be resistant to biotransformation or biodegradation is becoming smaller.

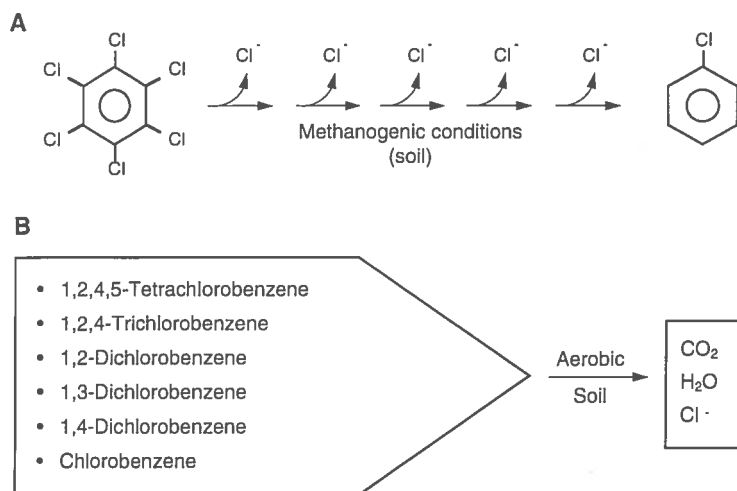


Figure 3. Reductive dehalogenation of chlorobenzenes under anaerobic conditions (A). Biodegradation (mineralization) of chlorobenzenes under aerobic conditions (B).

drocarbon-contaminated sites under consideration for bioremediation. Because such bacteria are ubiquitous, it is much more useful to assess their activity as revealed by the degradation of hydrocarbons or transformation of electron acceptors. The biology can be assumed to be present but limited by other factors. In contrast, bacteria able to degrade specific synthetic chemicals cannot be assumed to be widely distributed in the field. They might be present only in specialized ecosystems such as waste treatment systems at manufacturing sites or in the subsurface at contaminated sites. Detection of bacteria able to grow on specific compounds in contaminated sites and failure to detect them in nearby uncontaminated areas can be taken as strong evidence for natural attenuation. Thus, prediction of natural attenuation requires an understanding of whether capable bacteria evolve at a site or are transported to the site by dispersion. Absence of bacteria able to catalyze the degradation of compounds known to be biodegradable could provide an opportunity for bioaugmentation, a strategy that has earned a poor reputation because of misapplication in the past.

The third question is whether conditions appropriate for natural attenuation exist or can be created at the site. Issues of electron donors and acceptors, bioavailability, mass transfer, hydrogeology, contaminant mixtures, and concentration must be resolved. A good understanding of the biodegradation process including reaction stoichiometry and kinetics is essential for evaluation of the potential for natural attenuation. Fortunately, such understanding exists for a wide range of synthetic chemical contaminants.

Chloroaromatic Compounds

Bacteria able to degrade all but the most complex chloroaromatic compounds have been discovered during the past 20 years. Chlorobenzenes including hexachlorobenzene can be sequentially dehalogenated to chlorobenzene under methanogenic conditions in soil slurries (Figure 3) (Ramanand et al., 1993). Reductive dehalogenation of chlorobenzene has not been reported, but chlorotoluenes are dehalogenated to toluene in the above methanogenic systems and it seems likely that chlorobenzene could serve as a substrate for reductive dehalogenation.

Chlorobenzenes up to and including tetrachlorobenzene are readily biodegraded under aerobic conditions (Figure 3). Bacteria able to grow on chlorobenzene (Reineke and Knackmuss, 1984), 1,4-dichlorobenzene (Reineke and Knackmuss, 1984; Schraa et al., 1986; Spain and Nishino, 1987), 1,3-dichlorobenzene (de Bont et al., 1986), 1,2-dichlorobenzene (Haigler et al., 1988), 1,2,4-trichlorobenzene (van der Meer et al., 1987), and 1,2,4,5-tetrachlorobenzene (Sander et al., 1991) have been isolated and their metabolic pathways have been identified. The pathways for aerobic degradation are remarkably similar and lead to the release of the halogens as HCl. Chlorobenzenes are very good candidates for natural attenuation under either aerobic or anaerobic conditions. Aerobic bacteria able to grow on chlorobenzene have been detected at a variety of chlorobenzene-contaminated sites but

not at adjacent uncontaminated sites (Nishino et al., 1994), providing strong evidence that they are selected for their ability to derive carbon and energy from chlorobenzene degradation in situ. Removal of multiple halogens as HCl consumes a large amount of alkalinity and produces a considerable drop in the pH of unbuffered systems which could lead to a loss of microbial activity at some sites.

Chlorophenols and chlorobenzoates are dehalogenated under anaerobic conditions in sediments and subsurface material (Hagglblom and Valo, 1995; Suflita and Townsend, 1995). In some instances the dehalogenation clearly yields energy for the growth of the specific bacteria. In other examples the dehalogenation is specific and enriched in the community but has not been rigorously linked to energy production. Addition of small fatty acids or alcohols as electron donors or sources of carbon can enhance the process of reductive dehalogenation.

Aerobic pathways for the degradation of chlorophenols and chlorobenzoates are initiated by oxygenase-catalyzed attack on the aromatic ring and subsequent removal of the halogen after ring fission or hydrolytic replacement of the halogen with a hydroxyl group. Bacteria able to grow on chlorophenols and chlorobenzoates are widely distributed and are readily enriched from a variety of sources which indicates a high potential for natural attenuation. The chlorophenols are unusual among the synthetic compounds discussed here in that they can be very toxic to microorganisms. They are often used as biocides and, therefore, high concentrations can dramatically inhibit biodegradation. Inoculation with specific bacteria has been helpful in overcoming toxicity and stimulating degradation of chlorophenols (Hagglblom and Valo, 1995).

Pentachlorophenol deserves special consideration because it has been widely used as a wood preservative and has been released into the environment throughout the world. Reductive dehalogenation under methanogenic conditions can lead to mineralization (Hagglblom and Valo, 1995). Aerobic bacteria catalyze the replacement of the chlorine in the 4 position by a hydroxyl group to form tetrachlorohydroquinone. Subsequent reductive dehalogenations lead to the formation of ring fission substrates. Bacteria able to degrade pentachlorophenol are widely distributed, and both experimental and full-scale bioremediation projects have been successful in field applications (Hagglblom and Valo, 1995). Addition of selected strains has been helpful in some instances. In others, indigenous strains have been used. Wood treatment facilities typically are contaminated with complex mixtures of organic compounds; therefore, investigations of toxicity must be conducted for each site under consideration. Natural attenuation of pentachlorophenol seems to be possible, because specific bacteria able to use it as a growth substrate are enriched at contaminated sites. However, rates seem to be low at the sites investigated to date due to toxicity and bioavailability of the pentachlorophenol.

Polychlorinated biphenyls (PCBs) have been studied extensively because of their stability, toxicity, and bioaccumulation potential (Bedard and Quensen, 1995). Anaerobic transformation of PCBs (Figure 4) is catalyzed by bacteria

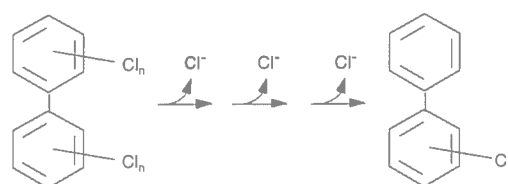


Figure 4. Anaerobic dechlorination of PCBs (Abramowicz, 1995; Bedard and Quensen, 1995).

Table 1. Sites where dechlorination has been detected (Abramowicz, 1995; Bedard and Quensen, 1995).

Contaminated Sites		Uncontaminated Sites
Escambia Bay Florida	Moreau Drag Strip New York	Adirondack Marsh New York
Fox River/Green Bay Wisconsin	New Bedford Harbor Massachusetts	Center Pond Massachusetts
Grass River New York	Otonabee River/Rice Lake Canada	Hudson River New York
Hoosic River Massachusetts	Rhine River Germany	Puget Sound Washington
Housatonic River Massachusetts	Rhine River The Netherlands	Red Cedar River Michigan
Hudson River New York	Sheboygan River Wisconsin	Saline River Michigan
Kalamazoo River Michigan	Silver Lake Massachusetts	St. Lawrence River New York
Lake Hartwell South Carolina	St. Lawrence River New York	
Lake Ketelmeer The Netherlands	Waukegan Harbor Illinois	
Lake Shinjii Japan	Woods Pond Massachusetts	

in aquatic sediment from a wide range of both contaminated and uncontaminated sites (Table 1). Higher activities in contaminated sites suggest that the dehalogenation reactions provide a selective advantage to the microbial population which indicates the potential for significant natural attenuation. A number of studies have clearly demonstrated that natural attenuation of PCB is taking place in anaerobic sediments at significant rates. Methanogenic conditions in freshwater sediments seem to provide the highest rates of reductive dehalogenation.

Dehalogenation converts the more highly chlorinated congeners to less-chlorinated products containing one to four

chlorines. Complete dehalogenation does not occur, but the depletion of the more highly chlorinated congeners dramatically reduces not only the toxic and carcinogenic potential, but also the bioaccumulation potential. A variety of dechlorination patterns have been identified as a function of the microbial community involved. The patterns are constant within a given microbial community or enrichment, which supports the premise that the dehalogenation provides a selective advantage to the organisms involved. The results also suggest that a wide range of bacteria have the ability to dehalogenate PCBs. The electron donors for the dehalogenation in sediment are unknown. Addition of exogenous carbon

sources does not stimulate the reaction. In contrast, "priming" the mixtures with low levels of bromobiphenyl or specific isomers of tetrachlorobiphenyl (Bedard et al., 1996; Bedard and Quensen, 1995) seems to selectively enrich a population of PCB-dechlorinating bacteria and dramatically stimulate the dechlorination of the other congeners.

The lower-chlorinated PCB congeners, whether part of the original Arochlor mixture or derived from reductive dehalogenation, are biodegraded by aerobic bacteria (Figure 5) (Flanagan and May, 1993). The initial attack is catalyzed by a 2,3- or 3,4-dioxygenase followed by a sequence of reactions that lead to ring cleavage and accumulation of chlorobenzoates that are readily degraded by a variety of bacteria. The enzymes that oxidize PCBs are produced by bacteria grown on biphenyl, and addition of biphenyl to slurry-phase reactors stimulates the growth and activity of PCB degraders. Such stimulation has been shown to be effective in the field (Harkness et al., 1993). There is also good evidence that aerobic PCB degradation is taking place in contaminated river sediments (Flanagan and May, 1993)

It seems clear that reductive dechlorination is ongoing at a wide range of PCB-contaminated sites. The strategy of anaerobic dehalogenation followed by aerobic degradation seems to be particularly effective with PCB whether in an engineered system or in natural systems such as would occur during resuspension of anaerobic sediments. To date, the complete biodegradation of PCB is slow and difficult to predict or control in the field. Several new strategies including construction of novel strains may increase the potential for effective PCB biodegradation.

Chloroaliphatic Compounds

Several good reviews have been published on the biodegradation of small (one and two carbon) chloroaliphatic compounds (Adriaens and Vogel, 1995; Fetzner and Lingens, 1994; Wackett, 1995), and these compounds were the major topic of a recent conference (U.S. EPA, 1996). Therefore, this paper only briefly mentions some aspects that might otherwise be overlooked. Among the one- and two-carbon chlorinated compounds, the more highly chlorinated molecules are subject to reductive dehalogenation under a variety of conditions. Thus, carbon tetrachloride can be sequentially reduced to chloro-

form and dichloromethane. Similarly, perchloroethylene can be reduced to ethylene via trichloroethylene, dichloroethylene, and vinyl chloride. The degradation of chloroethylenes has been discussed by Gossett and Zinder (1996). Most of the work to date has focused on mixed microbial cultures that use chlorinated solvents fortuitously as an electron acceptor. Such activity is very widely distributed in anaerobic ecosystems and catalyzes the slow and often partial reduction of chlorinated contaminants. In contrast, some microbial communities and a few isolated strains can derive energy from the use of chlorinated compounds as terminal electron acceptors. Such processes are much faster than the cometabolic processes because they provide a selective advantage for the bacteria and are self-sustaining.

Several chloroaliphatic compounds can serve as growth substrates for aerobic bacteria (Table 2). The chemicals that can support growth are excellent candidates for natural attenuation, whereas biotransformation is more difficult to predict. Biotransformation is most likely in situations where excess organic material is available to serve as an electron donor. The table was constructed based on a cursory review of the literature. An \times mark on Table 2 indicates only that the process has been reported and should not be taken to indicate that it will happen at all sites. Bacteria able to use the chemicals to support growth appear to be relatively rare, and little systematic work has been done to determine their distribution. The more-chlorinated compounds such as trichloroethylene and chloroform do not provide energy and carbon for aerobic growth although they can be transformed to harmless products. In contrast, methylene chloride can support the growth of both anaerobes (Magli et al., 1995) and aerobes (Fetzner and Lingens, 1994). Similarly, 1,2-dichloroethane (Stucki et al., 1983) and vinyl chloride (Fetzner and Lingens, 1994) can be readily degraded by aerobic bacteria. Vinyl chloride can also be mineralized under iron-reducing conditions (Bradley and Chapelle, 1996). Any of these compounds that serve as growth substrates would be excellent candidates for natural attenuation under conditions where oxygen is present. Aerobic mineralization of the related molecule, ethylene dibromide, has been reported (Frietas dos Santos et al., 1996; Pignatello, 1987), but the distribution of the responsible bacteria and the corresponding ability to predict degradation are not well understood.

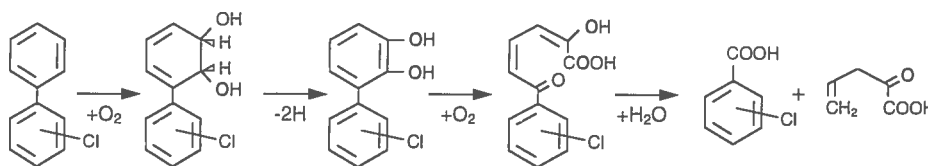


Figure 5. Aerobic degradation of chlorobiphenyls. No single strain is currently known to carry out the complete process, but all of the reactions have been demonstrated and the overall process is fairly common in PCB-contaminated rivers (Flanagan and May, 1993).

Table 2. Biodegradation and biotransformation of chloroaliphatic compounds.

Compound	Anaerobic		Aerobic	
	Transformation	Growth	Transformation	Growth
Carbon tetrachloride	X		X	
Chloroform	X		X	
Methylene chloride	X	X		X
Chloromethane	X		X	
Perchloroethane	X			
Trichloroethane	X			
1,2-Dichloroethane		X		X
Perchloroethylene	X	X		
Trichloroethylene	X	X	X	
Dichloroethylene	X	X	X	
Vinyl chloride	X		X	X
1,2-Dibromoethane	X			X

Nitroaromatic Compounds

The literature on biodegradation of nitroaromatic compounds has been reviewed recently (Spain, 1995a; Spain, 1995b). Nitroaromatic compounds are subject to reduction of the nitro groups in the environment under either aerobic or anaerobic conditions. Reduction does not lead to complete degradation in most instances and could be considered nonproductive for purposes of natural attenuation. In contrast, aerobic bacteria able to grow on nitrobenzene, nitrotoluenes, dinitrotoluenes, dinitrobenzene, nitrobenzoates, picric acid, and other nitrophenols have been isolated from a variety of contaminated sites, which suggests that natural attenuation is taking place at such sites. For example, Bradley et al. (1997) have recently measured mineralization of dinitrotoluenes in aquifer material from a dinitrotoluene-contaminated site. They concluded that the indigenous microorganisms provide a significant degradative capacity for the contaminant.

The simple nitroaromatic compounds can be considered excellent candidates for natural attenuation as long as the degradation process yields a selective advantage. Some of the compounds including 3-nitrophenol, nitrobenzene, 4-nitrotoluene, and 4-nitrobenzoate are degraded via catabolic pathways that minimize the use of molecular oxygen and are particularly well suited for operation in the subsurface where oxygen is limiting. The pathways all involve a partial reduction of the molecule prior to oxygenative ring fission. For example, the first three steps in the pathway for degradation of nitrobenzene can take place in the absence of oxygen (Spain, 1995a). Oxygen is required only for ring fission and subsequent metabolism.

Mixtures of the isomeric nitro compounds can be problematic for microbial degradation. For example, the industrial synthesis of polyurethane produces large amounts (U.S. production of 941 million pounds in 1995) of 2,4- and 2,6-dinitrotoluene in a ratio of 4:1. Bacteria able to grow on 2,4-dinitrotoluene have been studied extensively. Unfortunately,

2,6-dinitrotoluene inhibits the degradation of 2,4-dinitrotoluene and may prevent natural attenuation. Bacteria able to grow on 2,6-dinitrotoluene have been isolated recently (Nishino and Spain, 1996), and insight about the metabolic pathway might allow better prediction of degradation of the mixture.

Ketones

Acetone and other ketones are not xenobiotic compounds, but most of the current production is via synthetic routes. They are readily biodegraded by both aerobic and anaerobic (Janssen and Schink, 1995) bacteria in soil and have a very high potential for natural attenuation.

Methyl-*tert*-butyl Ether (MTBE)

Gasoline oxygenates such as ethanol, MTBE, and *tert*-butyl alcohol are used extensively as octane enhancers in unleaded gasoline. The ether bond of MTBE makes it particularly resistant to biodegradation. Its water solubility, low volatility, and high concentrations in gasoline (up to 15%) create concerns about its behavior in the subsurface. Preliminary studies indicate that it behaves almost as a conservative tracer in gasoline-contaminated sites (James Weaver, personal communication). Mixed cultures able to grow on MTBE have been enriched from refinery and chemical plant waste treatment systems (Cowan and Park, 1996; Salanitro et al., 1994), so it is clear that bacteria can successfully attack the ether bond. The degradation rates are slow, however, and there is no evidence that the bacteria are widely distributed in soil. MTBE and other oxygenates containing ether bonds biodegrade very slowly if at all under anaerobic conditions (Mormile et al., 1994). At present, even though the biological capability for MTBE degradation is known to exist, the potential for natural attenuation of MTBE seems low. The problem is sufficiently important to merit additional study, perhaps involving extensive acclimation of soil communities or

bioaugmentation. The available evidence indicates that *tert*-butyl alcohol is much more readily degradable than MTBE under aerobic or anaerobic conditions.

Nitrate Esters

A variety of nitrate esters including glycerol trinitrate, pentaerythritol tetranitrate, and nitrocellulose have been used extensively as explosives. Recent studies have indicated that the nitrate esters can be degraded by bacteria from a variety of sources (White and Snape, 1993; White et al., 1996). Bacterial metabolism releases nitrite which can serve as a nitrogen source and yield a selective advantage for the organisms. The biodegradation of nitrate esters has only recently been studied extensively and little is known about degradation in the environment. The recent laboratory results show considerable promise that natural attenuation is possible, but more information is needed on the bioavailability, toxicity, and kinetics of the process.

Pesticides

Most pesticides used in the past 20 years in the United States have been formulated to degrade in the environment, and a considerable amount of information is available on degradation kinetics in soil and water. The U.S. Environmental Protection Agency Risk Reduction Engineering Laboratory in Cincinnati, Ohio has developed an extensive Pesticide Treatability Database that contains information on a variety of compounds. Many pesticides hydrolyze and yield compounds that serve as growth substrates or sources of nitrogen or phosphorus for bacteria. Enhanced degradation of pesticides has been studied extensively (Racke and Coates, 1990) and

is closely related to natural attenuation. For example, carbamates (Topp et al., 1993), chlorophenoxyacetates (Fulthorpe et al., 1996), dinitrocresol (Jensen and Lathrup-Larsen, 1967), atrazines (Mandelbaum et al., 1995), and some organophosphates serve as growth substrates for bacteria and would be good candidates for natural attenuation. A variety of other pesticides are hydrolyzed by extracellular enzymes derived from soil bacteria but provide no advantage to the organisms that produce the enzymes. Similarly, some of the organohalogen insecticides can be reductively dehalogenated but provide no advantage to specific organisms. Their biodegradation rates are proportional to the biomass and activity in the soil. Other organohalogens, such as lindane, can serve as growth substrates for specific bacteria (Siddhartha et al., 1990), but such bacteria seem not to be widely distributed.

Conclusion

To date, the focus of natural attenuation has been on hydrocarbon fuels and chlorinated aliphatic solvents. A wide range of synthetic chemicals released in the environment are known to be biodegradable by bacteria (Figure 6). Much is known about the processes and their requirements. The potential for natural attenuation of biodegradable contaminants should be considered before more costly and disruptive treatment options. Detection of specific bacteria able to use the contaminant to support growth or demonstration of the activity of such organisms in material from the site can be taken as strong evidence that natural attenuation is taking place. This paper is a modified version of a paper presented at the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water held in Dallas, Texas, September, 1996 (Spain, 1996).

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|---|---|
| <ul style="list-style-type: none">• Hydrocarbons• Acetone• Methylene ketone• Chlorobenzene• Dichlorobenzenes• 1,2,4-Trichlorobenzene• 1,2,4,5-Tetrachlorobenzene• Chlorophenols• Pentachlorophenol• Methylene chloride• 1,2-Dichloroethane• Perchloroethylene• Trichloroethylene• Dichloroethylene | <ul style="list-style-type: none">• Vinyl chloride• 1,2-Dibromoethane• Polychlorinated biphenyls• Nitrobenzene• Nitrotoluenes• Dinitrotoluenes• 1,3-Dinitrobenzene• Nitrophenols• 2,4-Dinitrophenol• Picric acid• Nitrobenzoic acids• Nitroglycerin• Pesticides• Aniline |
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Figure 6. Compounds with potential for natural attenuation.

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