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ENVIRONMENTAL RESEARCH BRIEF

Anaerobic Biotransformation of Contaminants in the Subsurface

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Abstract

Anaerobic conditions predominate in contaminated aquifers and are not uncommon in noncontaminated areas. Comparatively little is known about degradative processes and nutrient cycling under anaerobic conditions. However, it is apparent these processes are fundamentally different and more complex than comparable aerobic processes. Research in this area is critical to our understanding of the fate of contaminants in the subsurface environment and for the design and operation of efficient and effective treatment technologies. The objective of this research brief is to report the current status of research directed toward defining anaerobic microbial metabolic processes which occur in the subsurface environment.

Introduction

Bioremediation technologies hold great promise as economical and permanent solutions for contaminants in the environment. This is particularly true for the terrestrial subsurface where technical or economic constraints may preclude physical removal of a contaminant and thus its treatment by sorption, incineration or containment methods. Bioremediation technologies have several other inherent advantages. Instead of transferring contaminants from one environmental medium to another, complete breakdown or mineralization of contaminants is often possible. Further, the partial transformation of a contaminant can sometimes make it more environmentally acceptable. Bioremediation is also usually cheaper than conventional physical or chemical treatment schemes.

To date, most subsurface biotreatment processes have relied on aerobic microbial metabolism. In these cases oxygen serves as a terminal electron acceptor for the microorganisms and may be supplied in various forms (compressed air, liquid oxygen, hydrogen peroxide or ozone). Such systems have shown great success in the cleanup of a wide variety of contaminants, but they can also suffer from several drawbacks. For example, some compounds, particularly halogenated solvents, are resistant to biodegradation under aerobic conditions. Also, most of the costs associated with aerobic bioremediation are due to the low solubility of molecular oxygen and the difficulties associated with the introduction and transport of this electron acceptor in the subsurface.

However, microbial metabolism also occurs in the absence of molecular oxygen, but there is a general lack of appreciation for the metabolic potential of anaerobic microorganisms in the subsurface. Historically, this may be attributed to the difficulties associated with research on anaerobic microorganisms (specialized equipment, slow growth rates, poorly defined growth requirements), the misconceptions about the numbers and activities of microorganisms in the subsurface, and the expense and technical limitations of sampling the subsurface environment.

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To a greater or lesser extent these problems have been addressed by the advancement of technology and the results of investigations into the subsurface environment. New methods and equipment for the isolation, cultivation, and manipulation of anaerobic microorganisms have made their study less of an art and more a set of widely used and accepted techniques. In recent years microbiological studies of the subsurface have demonstrated the abundance, diversity and important activities of bacteria in this environment. Technical developments have allowed the recovery of high quality microbiological samples from both the near and deep subsurface. Together these advances and discoveries have allowed us access to the subsurface and techniques for the characterization of the indigenous microorganisms. However, many questions surrounding anaerobic metabolic abilities remain, including: 1) What types of contaminants are susceptible to anaerobic decay and which are not? 2) What structural features of the contaminants favor its bioconversion under anaerobic conditions? 3) Are pollutants mineralized or only partially transformed? 4) What rates of transformation can be expected? 5) How do such transformations impact predictions of the transport and fate characteristics of contaminants?

To address some of these questions, researchers at the University of Oklahoma, through the National Center for Ground Water Research and the Robert S. Kerr Environmental Research Laboratory (RSKERL) of the U.S. EPA have been investigating anaerobic biotransformations of pollutant chemicals in material collected from the terrestrial subsurface. They have found an abundant and diverse microflora existing in anoxic aquifers. Further, the microorganisms within these aquifers are more metabolically diverse than originally believed. It appears that these organisms can catalyze biotransformation reactions which will be useful for refining the predictions of the transport and fate of contaminants and possibly form the basis for novel bioremediation strategies.

Metabolic Principles

Heterotrophic organisms (like humans and most bacteria) oxidize organic compounds to obtain energy. In this process, electrons or reducing equivalents from the oxidizable organic compound (substrate) are transferred to and ultimately reduce an electron acceptor. The electron acceptor may be an organic or inorganic compound. During this electron transfer process, usable energy is recovered through a complex series of oxidation-reduction (redox) reactions by the formation of energy storage compounds or electrochemical gradients. The oxidation of organic compounds coupled to the reduction of molecular oxygen is termed aerobic heterotrophic respiration (Table 1).

When oxygen is unavailable, redox reactions can still occur. In anaerobic respiration, the oxidation of organic matter can be coupled with a number of other organic or inorganic electron acceptors. Some microorganisms carry out a process known as fermentation. Fermenting organisms utilize their substrate as both an electron donor and acceptor. In this process an organic compound is metabolized with a portion of that compound becoming a reduced end product and another becoming an oxidized product. A common example of this process is the alcoholic fermentation of starch to CO_2 (oxidized product) and ethanol (reduced product). Fermentative organisms play a critical role in anaerobic consortia by transforming organic substrates into simple products which can then be used by other members of the community. Still other organisms can utilize

Table 1. Selected types of aerobic and anaerobic respiration involved in the microbial metabolism of organic matter.

Process	Electron Acceptor	Metabolic Products	Relative Potential Energy
Aerobic Heterotrophic Respiration	O_2	CO_2, H_2O	HIGH
Denitrification	NO_3^-	CO_2, N_2	
Iron Reduction	Fe^{3+}	CO_2, Fe^{2+}	
Sulfate Reduction	SO_4^{2-}	CO_2, H_2S	
Methanogenesis	CO_2	CO_2, CH_4	LOW

alternate electron acceptors (Table 1). The potential energy available from the oxidation of a particular substrate coupled with the reduction of different electron acceptors varies considerably. A higher energy yielding process will tend to predominate if the required electron acceptor is available. Under anaerobic conditions, microorganisms may enter into very tightly linked metabolic consortia. That is, the catalytic entity responsible for the destruction of a contaminant is often not a single type of microorganism. Such consortia can develop regardless of the nature of the terminal electron acceptor (see below).

Impact of Contaminants on Subsurface Ecology

When readily degradable organic matter enters the subsurface in sufficient quantities, it can produce a variety of chronologically and spatially defined metabolic zones (Figure 1). These zones are not necessarily mutually exclusive and are dependent on the availability of electron acceptors. As organic matter enters an oxygenated aquifer, whether it is a human produced (anthropogenic) contaminant or an influx of "natural" material, indigenous aerobic heterotrophic microorganisms metabolize the organic material and consume the available oxygen in the process. When this occurs, aerobic respiration slows and eventually stops. This allows for the development of anaerobic metabolic communities. If nitrate is available, dissimilatory nitrate-reduction (denitrification) tends to become the dominant metabolic process linked to the degradation of organic contaminants. Sulfate tends to serve as the electron acceptor (sulfate reduction) when nitrate is depleted. Sulfate reduction may lead to the formation of sulfides. Organic matter can still be consumed when sulfate is depleted by coupling its metabolism with the reduction of CO_2 in the process of methanogenesis (Figure 1). The metabolic processes for the consumption of organic matter illustrated in Table 1 and Figure 1 are far from complete. Other electron acceptors such as iron or manganese may also participate in this respect. Due to the abundance of oxidized iron (Fe^{3+}) in the subsurface and the apparent catabolic diversity of the organisms responsible for iron reduction (Lovely and Lonergan, 1990), the environmental importance of such reactions may be quite considerable. There is no reason to suspect that the biodegradation potential in different metabolic zones will necessarily be similar. This potential will be based on the energetics associated with the dominant redox processes,

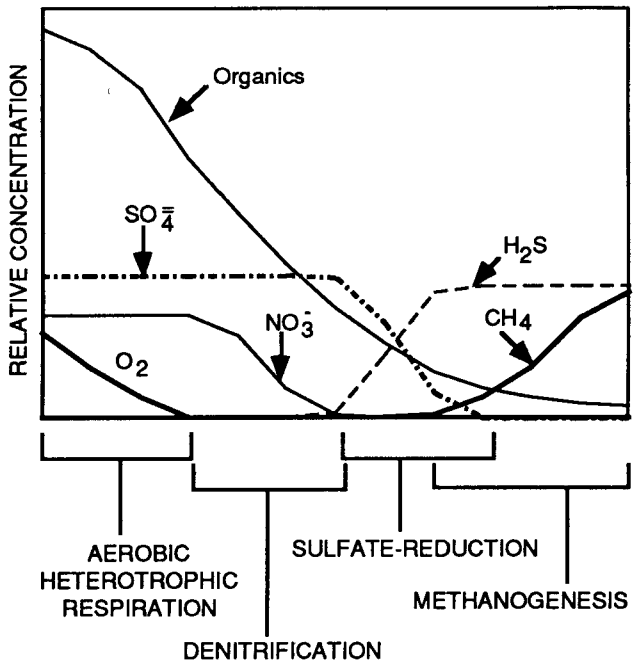


Figure 1. Changes in Chemical Species and Microbial Processes in Contaminated Subsurface Material. (adapted from Bouwer and McCarty, 1984)

the metabolic diversity of the microbial communities, the immediate geochemical conditions and the chemical nature of the contaminant of concern.

In the last 20 years, fundamental developments in the theory, microbiological techniques, and understanding of anaerobic processes have occurred. The impact of these developments has been for researchers to reevaluate the types of chemicals that are subject to biodegradation under anaerobic conditions. In only the last 10 years, several new anaerobic processes such as reductive dehalogenation and anaerobic alkyl benzene degradation have been identified. The future will undoubtedly harbor even more exciting and significant developments. However, a glimpse of future progress must be firmly rooted in an assessment of the current state of the art. The following subsections will hopefully serve to help provide such an assessment.

Anaerobic Biotransformations

The metabolic pathways utilized by indigenous subsurface microorganisms for the degradation of environmental contaminants and natural analogs is an area of intense ecological research. As noted above, under anaerobic conditions most organic compounds are degraded by groups of interacting microorganisms referred to as a consortium. In the consortium, individual types of organisms carry out different specialized reactions which, when combined, can lead to the complete mineralization of a particular compound. The metabolic interaction between organisms can be complex and may be so tightly linked under a given set of conditions that stable consortia have been mistakenly identified as a single species. Without all the individual members of the consortium, the degradation of the initial substrate tends to be inhibited. In a hypothetical sulfate-reducing consortium growing on toluene, the sulfate-reducing bacteria would probably not be oxidizing the toluene directly, but rather a product of a primary or secondary transformation. Together, the reactions tend to be pulled in the forward direction. An example of the complexity of a methanogenic consortium is shown in Figure 2.

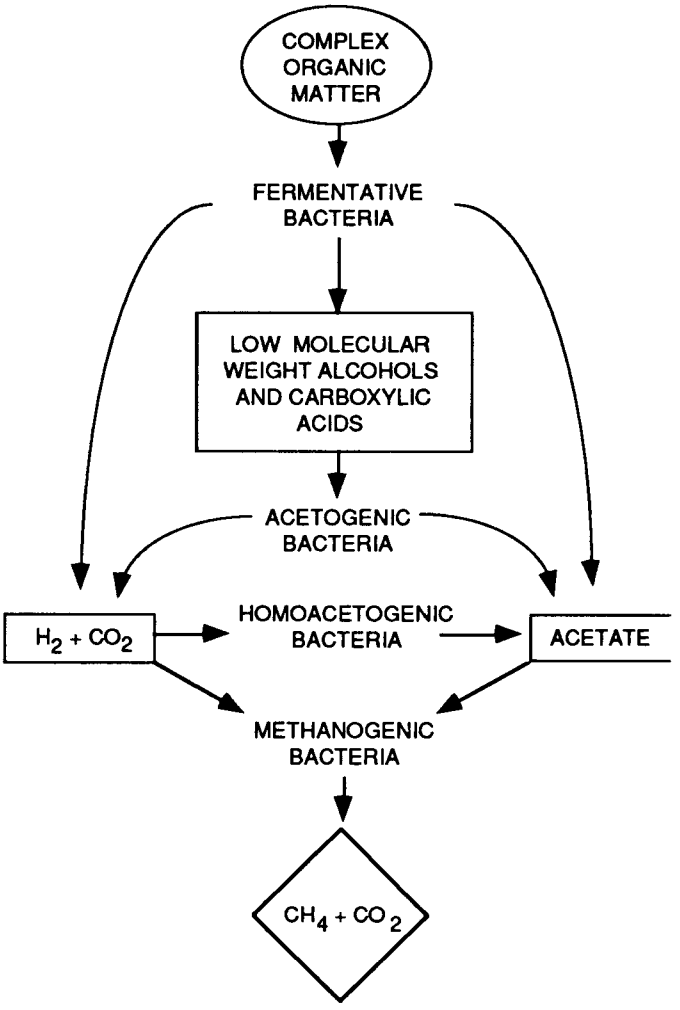


Figure 2. Degradation of Organic Material by Methanogenic Consortia. (adapted from Schink, 1988)

There seems to be several advantages to the evolution of microbial consortia: 1) This arrangement allows for the creation of microenvironments where certain types of organisms can survive in otherwise hostile conditions. 2) Reactions that are thermodynamically unfavorable can be driven by favorable reactions when they are metabolically linked within the consortium. 3) Toxic or inhibitory compounds may be removed by resistant members. 4) This system takes advantage of the diverse metabolic capabilities of microorganisms by allowing for the

formation and enrichment of associations that can utilize an introduced nutrient much faster than a given species could evolve a novel complex degradation pathway.

Aromatic and Oxygen-Substituted Aromatic Compounds

Aromatic hydrocarbons are among the most common ground water contaminants. Although the aerobic biodegradation of alkylbenzenes has been extensively studied, until recently anaerobic degradation of these compounds was considered unlikely (Wilson and McNabb, 1983). New research has shown that alkylbenzenes such as toluene are degraded under nitrate-reducing (Kuhn et. al. 1988), methanogenic (Wilson et.al. 1986) and iron-reducing conditions (Lovely and Lonergan, 1990). Anaerobic metabolism of toluene appears to require the oxidation of the methyl group or ring with water serving as the source of oxygen atoms (Grbic-Galic and Vogel, 1986; Vogel and Grbic-Galic, 1986). Oxygen-substituted aromatic compounds are important ground water contaminants in their own right as well as intermediates of anaerobic alkylbenzene degradation. Phenol, cresol and benzoic acid appear to degrade under several anaerobic systems. Table 2 summarizes reports of degradation of aromatics and oxygen-substituted aromatics in soils and subsurface material under different anaerobic conditions. Under aerobic conditions, aromatic degradative pathways tend to feed into a few common intermediates such as catechol and protocatechuic acid. From the information available at this time, it appears that a similar situation may also occur under anaerobic conditions. Benzoic acid appears as an intermediate in numerous anaerobic aromatic degradation pathways.

Oxygen, Nitrogen, and Sulfur Heterocyclic Compounds

Two thirds of the approximately 4 million known organic compounds have a chemical structure made up of carbon, and either oxygen, nitrogen or sulfur atoms arranged in a ring. These compounds are referred to as heterocycles and are widely used components of, or precursors for, the synthesis of pharmaceuticals, pesticides, explosives, dyes and food additives. These compounds are also found naturally in fossil fuel deposits and serve in important metabolic roles (such as vitamins, nucleic acids, proteins and carbohydrates) in living systems.

Many of the heterocyclic compounds which are found as ground water contaminants are derivatives of pyridines, furans and thiophenes, but a few saturated heterocycles such as dioxane are also of concern. Encouragingly, most of these derivatives were anaerobically biotransformed under methanogenic or sulfate-reducing conditions (Table 3). Substitution of a carboxyl group increased the biodegradability of the O, N, and S heterocycles. These results are similar to those reported for non-heterocyclic aromatic compounds. In general it appears that oxygen and nitrogen heterocyclic compounds are more susceptible to anaerobic degradation than those containing a sulfur heteroatom.

Nitrogen-Substituted and Sulfonated Benzenes

Substituted anilines, benzenesulfonamides and benzamides are frequent aquifer contaminants. The methylated or higher alkylated derivatives of these compounds are of particular importance. In 1987, the annual production of aniline alone exceeded 900 million pounds. Recent research has shown that the amino substituted benzenes were relatively easily anaerobically biodegraded when the aromatic nucleus was substituted with a carboxyl group (Table 4). This is in contrast to aniline and the methylated anilines (toluidines) which proved recalcitrant under methanogenic conditions. There were some indications of anaerobic biodegradation of m-toluidine and perhaps aniline itself under sulfate-reducing conditions, but the evidence is not strong. In contrast, aminobenzoic acids can be readily degraded under nitrate-reducing, sulfate-reducing and methanogenic conditions.

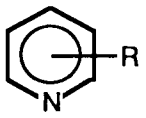
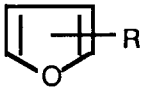
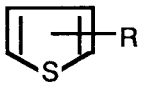
Benzamides were biodegraded under sulfate-reducing and methanogenic conditions (Table 5). When properly positioned, methyl group substituents did not render these derivatives resistant to biodegradation. However, multiple methyl substitutions or complicated alkylation patterns severely inhibited anaerobic decay (Table 5). Similarly, as a class of compounds, the aryl sulfonates tend to resist biodegradation under sulfate-

Table 2. Reported anaerobic biotransformations of aromatic compounds by subsurface microorganisms or with pure cultures.

Compound	Inoculum Source[a]	Incubation Conditions[b]	Reference
Toluene	AqM	M	Wilson et al., 1986.
Toluene, Xylenes	AqM	NR	Kuhn et al., 1988.
Toluene	PC	NR,IR	Lovely and Lonergan, 1990.
Toluene, Xylenes	AqM	NR	Hutchins et al., 1990.
Cresols	AqM	M,SR	Smolenski and Suflita, 1987.
Phenol, Benzoate, Hydroxybenzoate	AqM	M,SR,NR	Kuhn et al., 1989.
Phenol, Cresol, Benzoate, Hydroxybenzoate	PC	IR,NR	Lovely and Lonergan, 1990.
Phenoxy-acetate	AqM	M	Gibson and Suflita, 1986.
Methoxy-benzoate	PC	An	DeWeerd et al., 1988.
Hydroxy-biphenyl	AqM	SR	Suflita et al., 1990.

[a] Source of microorganisms in laboratory biotransformation experiments; AqM- Aquifer material, PC- Model Pure culture.
[b] Incubation conditions; M- Methanogenic, SR- Sulfate-reducing, NR- Nitrate-reducing, IR- Iron-reducing, An- Anaerobic (Fermentation).

Table 3. Anaerobic biotransformation of heterocyclic compounds under methanogenic conditions.

Compound	Chemical structure	R	Biotrans-formation[a]
Pyridine		H	+
4-Picoline		CH_3	+
3-Picoline		CH_3	-
2-Picoline		CH_3	-
Nicotinic acid		COOH	+
Furan		H	+
2-Methylfuran		CH_3	-
2-Furoic acid		COOH	+
Thiophene		H	-
2-Methylthiophene		CH_3	-
3-Methylthiophene		CH_3	-
2-Thiophene carboxylic acid		COOH	+

[a]Loss of test compound and production of methane relative to abiotic controls during 8 month anaerobic incubation in aquifer derived methanogenic microcosms. Adapted from Kuhn and Suflita, 1989c.

Table 4. Anaerobic biotransformation of amino-substituted benzenes in aquifer microcosms.

Compound	R group	Biotransformation[a] M[b]	Biotransformation[a] SR[c]
Aniline	H	-	+
o-Toluidine	CH_3	-	-
m-Toluidine	CH_3	-	+
p-Toluidine	CH_3	-	-
o-Aminobenzoate	COOH	+	+
m-Aminobenzoate	COOH	+	+
p-Aminobenzoate	COOH	+	+

[a] Loss of target compound relative to abiotic controls during 10 month anaerobic incubation.
[b] Methanogenic aquifer microcosms.
[c] Sulfate-reducing aquifer microcosms.
Adapted from Kuhn and Suflita, 1989a.

reducing or methanogenic conditions (Table 6). The only exception was the carboxyl substituted compounds (p-benzosulfonic acid) which was only partially transformed. Benzenesulfonamide and the aryl methyl benzenesulfonamide (p-toluenesufonamide) were degraded under methanogenic conditions. As in the case of benzamides, complicated patterns of alkylation inhibited anaerobic biotransformation.

Table 5. Anaerobic biotransformations of alkylated benzamides in aquifer microcosms.

Compound	Substituents R_1	R_2	R_3	Biotransformation[a] M[b]	Biotransformation[a] SR[c]
Benzamide	H	H	H	±	+
N-Methyl-benzamide	H	H	CH_3	±	+
N,N-Dimethyl-benzamide	H	CH_3	CH_3	-	-
p-Toluamide	CH_3	H	H	+	+
N,N-Diethyl-m-toluamide	CH_3	C_2H_5	C_2H_5	-	-

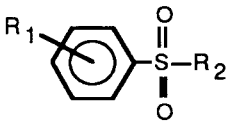
[a] Loss of target compound relative to abiotic controls during 11 month anaerobic incubation.
[b] Methanogenic aquifer microcosms.
[c] Sulfate-reducing aquifer microcosms.
Adapted from Kuhn and Suflita, 1989a.

Halogenated Compounds

Chlorinated aliphatic and aromatic compounds are undoubtedly some of the most pervasive and troubling ground water contaminants known. Such compounds may be toxic in low concentrations, carcinogenic and tend to resist aerobic degradation. However, recent research has shown that anaerobic microorganisms can transform many such compounds. They do so by catalyzing a reductive dehalogenation process (Suflita et al. 1982). During a reductive dehalogenation reaction, a chlorinated compound (for instance) acts as an electron acceptor in a novel type of anaerobic microbial respiration. The chloride moiety is removed from the molecule and replaced by a hydrogen (Vogel et al., 1987). Such a process usually renders the resulting compound more susceptible to subsequent transformations. Of course this reduction must be coupled to the oxidation of an electron donor. Recent research suggests that the required reducing equivalents may be supplied in a variety of forms (Gibson and Suflita, 1990). With chlorinated aromatics, in some cases, the compound can serve as both donor and acceptor, but with other compounds such as chloroethenes another source of reducing equivalents must be supplied. For their part, the bacteria likely gain energy during the process of reductive dehalogenation. Such reactions then form a promising scientific foundation upon which to build novel bioremediation strategies.

Chlorinated aromatic compounds are widely used components or synthetic precursors of solvents, pesticides, plastics and many other products of modern society. To date, over 30 different mono-, di-, tri-, and tetrahalogenated aromatic compounds have been tested for their susceptibility for anaerobic decay in aquifer microcosms. These compounds belonged to several chemical classes including the benzoates, phenols, phenoxyacetates, anilines, and a nitrogen heterocyclic compound. The list included numerous priority pollutants, several pesticides (2,4-D; 2,4,5-T;

Table 6. Anaerobic biotransformation of benzenesulfonic acids and benzenesulfonamides in aquifer microcosms.



Compound	Substituents R_1	Substituents R_2	Biotransformation[a] M[b]	Biotransformation[a] SR[c]
Benzenesulfonic acid	H	OH	-	-
Orthanilic acid	$NH_2(o)$	OH	-	-
Metanilic acid	$NH_2(m)$	OH	-	-
Sulfanilic acid	$NH_2(p)$	OH	-	-
p-Toluenesulfonic acid	CH_3	OH	-	-
p-Phenolsulfonic acid	OH	OH	-	-
p-Benzosulfonic acid	COOH	OH	+	+
Benzene-sulfonamide	H	NH_2	±	-
p-Toluene-sulfonamide	CH_3	NH_2	-	-
N-Methylbenzene-sulfonamide	H	$NHCH_3$	-	-
N,N-Diethyl-*p*-toluene-sulfonamide	CH_3	$N(C2H5)_2$	-	-
N-Ethyl-*p/m*-toluene-sulfonamide	CH_3	$NH(C_2H_5)$	-	-

[a] Loss of target compound relative to abiotic controls during anaerobic incubation (13 months for benzenesulfonic acids and 10 months for benzenesulfonamides).
[b] Methanogenic aquifer microcosms
[c] Sulfate-reducing aquifer microcosms.
Adapted from Kuhn and Suflita, 1989a.

bromacil) and chemicals known to be recalcitrant under aerobic conditions. Of the haloaromatic test chemicals, only four proved recalcitrant in anoxic aquifer microcosms. The others were reductively dehalogenated. For most compounds, the dehalogenation process continued in a sequential manner until all halogens were removed. Others were converted to products that were more susceptible to subsequent aerobic microbial metabolism. However, it is important to note that reductive dehalogenation reactions were confined to methanogenic microcosms and did not occur in sulfate-reducing microcosms.

To illustrate with a model compound, when 2,4,5-trichlorophenoxyacetate (2,4,5,-T) was incubated in aquifer sample from a methanogenic site and from a nearby sulfate-reducing site, the pesticide was dehalogenated only in the former incubations (Gibson and Suflita, 1990). Additional studies indicate that the requisite dehalogenating microorganisms were present at the sulfate-reducing site, but their metabolic activity was controlled, at least in part, by the high levels of sulfate (Gibson and Suflita, 1986). In consistent fashion, the addition of sulfate to microcosms made from the methanogenic aquifer material severely inhibited but did not completely preclude the dehalogenation of 2,4,5-T. The same types of findings were obtained when other haloaromatic compounds were similarly examined (Kuhn et al., 1990; Kuhn and Suflita, 1989b).

Slightly contrasting findings were obtained when the fate of halogenated aliphatic compounds were examined in comparable aquifer microcosms. Halogenated aliphatic compounds are widely used as cleaning and degreasing agents. Chloroethenes are among the most common and tightly regulated of ground water contaminants. Comparisons of the fate of tetrachloroethene (PCE) in methanogenic and sulfate-reducing aquifer systems indicate that PCE and trichloroethene (TCE) undergo reductive dechlorination under both conditions (Suflita et al., 1988). The reductive dechlorination of TCE and PCE led to the formation of intermediates that were more susceptible to aerobic microbial metabolism. However, while PCE and TCE are suspected carcinogens, the dehalogenated intermediate vinyl chloride (monochloroethene) is a known carcinogen. This compound also tends to be more mobile in the subsurface than the higher chloroethenes. This fact serves to illustrate the importance of transport and fate studies for accurate assessment of environmental risk and damage. The additional dehalogenation of vinyl chloride would eliminate this risk and has been shown (Vogel and McCarty, 1985; Freedman and Gossett, 1989). However, additional research on the environmental factors and microorganisms which influence reductive dechlorination processes is needed before anaerobic bioremediation of such compounds is safe and feasible.

Table 7 is a selected list of halogenated compounds known to be susceptible to anaerobic biotransformation reactions in soil, subsurface material, or by pure cultures of microorganisms.

Extrapolation of Metabolic Information

Studies with subsurface microorganisms or microcosms are extremely useful for exploring the limits of anaerobic biodegradation potential. It is, of course, important to question whether information generated in this fashion is environmentally meaningful. Yet microcosms have proven themselves repeatedly as tools for conducting scientifically rigorous experiments with limited quantities of subsurface material. That is, they afford an opportunity to conduct replicate trials employing appropriate negative and positive controls, while avoiding field contamination.

Moreover, microcosms can help researchers and regulators anticipate whether anaerobic biotransformation is likely, the conditions under which biodegradation occurs, the metabolic intermediates that may arise and the ultimate end products. As such, microcosms can assist researchers in their understanding of field contamination incidents. Laboratory studies are important in identifying new or unproven degradative processes and in defining the environmental factors that serve to retard or stimulate such reactions. The ability to make such determinations is independent of the inoculum source. Thus it matters little if subsurface microorganisms prove to be novel or unusual. Microbial metabolism tends to be a unifying feature of life and

Table 7. Reported haloorganic anaerobic biotransformations in subsurface- derived microcosms and pure culture.

Compound	Inoculum Source[a]	Incubation Conditions[b]	Reference
Cl_{1-2}-Phenols Cl_{1-2}-Benzoates Cl_{2-3}-Phenoxyacetates	AqM	M	Suflita et al., 1988.
Cl_{2-4}-Anilines	AqM	M,SR	Kuhn et al., 1990.
Bromacil	AqM	M	Adrian and Suflita,1990.
Tetrachloroethene, Trichloroethene (PCE, TCE)	AqM,PC	M,SR	Suflita et al., 1988.
PCE, TCE	PC	M	Fathepure et al., 1987.
TCE *cis* 1,2-Dichloroethene *trans* 1,2-Dichloroethene 1,1-Dichloroethene 1,2-Dibromoethane	AqM	M	Wilson et al., 1986.
Tetrachloromethane 1,1,1-Trichloroethane (CT, TCA)	AqM	An	Parsons et al., 1985
Trichloromethane (CF) TCA, CT	PC	An	Gälli and McCarty, 1989.
1,2-Dichloroethane, CT	PC	M,SR	Egli et al., 1987.
Bromoform, CF, CT	PC	M	Mikesell and Boyd, 1990.
Vinyl Chloride	AqM	An	Barrio-Lage et al., 1990.

[a] Source of microorganisms in laboratory biotransformation experiments; AqM- Aquifer material, PC- Model Pure culture.
[b] Incubation conditions; M- Methanogenic, SR- Sulfate-reducing, An- Anaerobic (Undefined).

factors which influence the degradation of contaminants in one ecosystem, whether on the surface or subsurface, will likely also impact others. This is demonstrated in studies (Gibson and Suflita, 1986) in which methanogenic and sulfate-reducing aquifer systems are compared to sewage sludge and pond sediments for the degradation of a number of aromatic and chloroaromatic compounds. For the most part, general agreement was observed, but sludge generally proved to be a poor surrogate inoculum for aquifer sediments.

Lastly, microcosms inherently incorporate the important site-specific variables that influence the success or failure of treatment schemes. To be sure, the mere demonstration that a particular degradative process can occur under certain ecological conditions is no guarantee that it will occur at a given subsurface location. However, the more environmentally realistic the microcosm, the greater the ease in extrapolating the resulting information from the laboratory to the field. Certainly, microcosms allow for the identification of environmental compartments where conditions limit or preclude degradative processes. Once the limiting factors are identified, microcosms provide experimental opportunities to try and overcome these limitations and to effectively stimulate desirable metabolic transformations.

In situ Anaerobic Bioremediation

The public and scientific community's increasing awareness of the impact of contaminants on the environment has been the impetus for the development and usage of bioremediation type technologies for the treatment of contaminants. This awareness notwithstanding, realization of the costs associated with alternate remedial measures also stimulates these biotechnological developments.

Nitrate-reducing based *in situ* bioremediation of petroleum-based fuels, which has been in limited use for a number of years, has recently undergone a rigorous field evaluation by RSKERL personnel (Hutchins et al. 1989). Early results suggest that this approach appears to be a viable technology. A field evaluation of the "natural" anaerobic biodegradation processes in a gasoline-contaminated aquifer is currently being conducted under the direction of Dr. John T. Wilson (RSKERL). It may be that the methanogenic fermentation of such contaminants will ultimately prove a useful treatment technology.

For chlorinated compounds anaerobic bacterial remediation designs may prove to be the most cost effective measure available. Such approaches are among the few to actually reduce the mass of such contaminants *in situ*. In low-permeability aquifers, the indigenous anaerobic population may be amenable to stimulation by introduction of only small doses of electron donors (or other required growth factors) and still produce the desired effects. Anaerobic treatment processes may not require the alteration of the *in situ* redox conditions in aquifers contaminated with complex waste mixtures. Hence, the addition of oxygen with its inherent problems, limitations and costs can be avoided. If we can learn to control and harness the reductive dechlorination process in an effective *in situ* technology, it will likely prove to have numerous advantages over existing aerobic bioremediation and physical-chemical treatment methods.

Conclusion

Research has shown that anaerobic microorganisms are much more metabolically versatile than originally believed. Indeed the major differences between anaerobic and aerobic systems may not be in whether a particular compound will degrade but what pathway is involved, which terminal electron acceptor is used, and what rates of bioconversion can be expected. Results summarized here are applicable to fate and transport models, assimilative capacity studies and biotreatment design considerations. Although the list of compounds that are known to be degradable under anaerobic conditions has increased greatly in recent years and steady progress has been made in delineating the appropriate metabolic pathways, the study of the microbial ecology of the subsurface, below the rhizosphere, is still in its infancy. This information gap limits our ability to

extrapolate these results without careful site-specific investigations. These considerations serve to identify subsurface ecology as a prime candidate for core research.

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Quality Assurance Statement

All research projects making conclusions or recommendations based on environmentally related measurements and funded by the Environmental Protection Agency are required to participate in the Agency Quality Assurance Program. This project was conducted under an approved Quality Assurance Program Plan. The procedures specified in this plan were used without exception. Information on the plan and documentation of the quality assurance activities and results are available from the Principal Investigator.