Microbial populations in contaminant plumes

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Abstract Efficient biodegradation of subsurface contaminants requires two elements: (1) microbial populations with the necessary degradative capabilities, and (2) favorable subsurface geochemical and hydrological conditions. Practical constraints on experimental design and interpretation in both the hydrogeological and microbiological sciences have resulted in limited knowledge of the interaction between hydrogeological and microbiological features of subsurface environments. These practical constraints include: (1) inconsistencies between the scales of investigation in the hydrogeological and microbiological sciences, and (2) practical limitations on the ability to accurately define microbial populations in environmental samples. However, advances in application of small-scale sampling methods and interdisciplinary approaches to site investigations are beginning to significantly improve understanding of hydrogeological and microbiological interactions. Likewise, culture-based and molecular analyses of microbial populations in subsurface contaminant plumes have revealed significant adaptation of microbial populations to plume environmental conditions. Results of recent studies suggest that variability in subsurface geochemical and hydrological conditions significantly influences subsurface microbial-community structure. Combined investigations of site conditions and microbial-community structure provide the knowledge needed to understand interactions between subsurface microbial populations, plume geochemistry, and contaminant biodegradation.

Résumé La biodégradation efficace des polluants souterrains requiert deux éléments: des populations microbiennes possédant les aptitudes nécessaires à la dégradation, et des conditions géochimiques et hydrologiques souterraines favorables. Des contraintes pratiques sur la conception et l'interprétation des expériences à la fois en microbiologie et en hydrogéologie ont conduit à une connaissance limitée des interactions entre les phénomènes hydrogéologiques et microbiologiques des environnements souterrains. Ces contraintes pratiques sont dues à des contradictions entre les échelles d'étude de l'hydrogéologie et de la microbiologie et à des limitations pratiques sur la capacité à définir avec précision les populations microbiennes dans les échantillons. Cependant, des progrès dans l'application de méthodes d'échantillonnage à l'échelle locale et des approches pluridisciplinaires des études de terrain ont commencé à améliorer de façon significative notre compréhension des interactions hydrogéologiques et microbiologiques. De plus, les analyses moléculaires et sur les cultures des populations microbiennes présentes dans les panaches de pollution souterraine ont mis en évidence une adaptation significative de ces populations aux conditions environnementales du panache. Les résultats d'études récentes laissent penser que la variabilité des conditions géochimiques et hydrologiques souterraines influence significativement la structure des communautés microbiennes souterraines. Des recherches combinées sur les conditions de terrain et sur la structure des communautés microbiennes apportent les informations nécessaires à la compréhension des interactions entre les populations microbiennes souterraines, la géochimie du panache et la biodégradation du polluant.

Resumen Para que la biodegradación de los contaminantes en el subsuelo sea eficiente se requiere: (1) una población microbiana con capacidad de degradación y (2) unas condiciones hidrológicas y geoquímicas favorables. Las restricciones de tipo práctico en los diseños y la interpretación de experimentos, tanto hidrogeológicos como microbiológicos, han dado lugar a un conocimiento limitado de la interrelación entre estas dos ciencias por lo que respecta al subsuelo. Estas restricciones incluyen: (1) inconsistencias entre las escalas de investigación en ambas ciencias (hidrogeología y microbiología) y (2) limitaciones prácticas para definir poblaciones microbianas en las muestras. Sin embargo,

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los avances en la aplicación de métodos de muestreo a pequeña escala y las investigaciones de campo con equipos interdisciplinares están mejorando significativamente el conocimiento de las interacciones entre hidrogeología y microbiología. Del mismo modo, los análisis moleculares y de cultivos sobre poblaciones microbianas en penachos contaminados han mostrado la adaptación de los microbios a las condiciones naturales. Estudios recientes sugieren que la variabilidad en las condiciones geoquímicas e hidrogeológicas del subsuelo afecta enormemente la estructura de la comunidad microbiana. Las investigaciones que combinan las condiciones del medio con la estructura de la comunidad microbiana proporcionarán el conocimiento necesario para entender las complejas relaciones entre las poblaciones microbianas subsuperficiales, la geoquímica de los penachos de contaminación y la biodegradación de los contaminantes.

Key words microbial processes · contamination · bioremediation · natural attenuation · heterogeneity

Introduction

Subsurface contaminant plumes comprise environments in which many microbial processes are accelerated. Sharp geochemical and biodegradative gradients in these plumes provide contrasting conditions for field and laboratory studies (Smith 1997). Because of the practical importance of contaminant biodegradation in the subsurface, some contaminant plumes have been studied intensively and for long periods of time (e.g., Madsen et al. 1996; Smith et al. 1996; Bekins et al. 1999; Ludvigsen et al. 1999). These studies serve as models for general understanding of subsurface microbial ecology. Some studies address how microbial diversity and abundance influence subsurface processes. They examine how unique aspects of microbial metabolism, genetic structure, and community organization can influence degradation processes. Other studies have examined how environmental controls influence microbial-community structure and function. Recent results suggest that variability in subsurface geochemical and hydrologic conditions significantly influences the subsurface microbial-community structure. Variations in the rate, mechanism, and completeness of biodegradative processes from one site to another indicate that increased understanding of the subsurface as a microbial habitat is needed to achieve a practical categorization of sites in terms of their remediation potential. A high level of detail and an interdisciplinary approach are required to address these issues.

Efficient biodegradation of subsurface contaminants requires (1) the presence of microbial populations with the necessary degradative capabilities, and (2) favorable subsurface geochemical and hydrologic conditions. The purpose of this paper is to review current understanding of how microbial and environmental interactions affect biodegradation in contaminant plumes. Thus, two themes are addressed: (1) adaptation of microbial communities to contaminant plumes, and (2) the influence of subsurface conditions on microbial processes. The review is restricted to long-term investigations of contaminated sites and contaminants for which multiple studies have been conducted. The focus is on non-manipulated systems and experiments that were conducted either in situ or under conditions representative of a site. By concentrating on these types of studies, the relations between subsurface environmental variables and the nature and activities of microorganisms in contaminant plumes are examined. Increased understanding of how microorganisms respond to subsurface conditions is needed to interpret variations in biodegradation rate and extent at each site. Eventually, knowledge of processes controlling subsurface microbial activity could be used to evaluate the reasons for success or failure of bioremediation at individual sites. Ideally, broad groupings of biodegradative capacity of subsurface environments is possible on the basis of geological, hydrological, and microbiological data. This information is also expected to be important in the design of adequate long-term monitoring schemes or development of bioaugmentation methods for plume remediation.

Background

Knowledge of microbial processes in the subsurface draws heavily from almost a century of investigations of soil and aquatic microbiology and wastewater treatment (Ford 1993; Hurst et al. 1997). Subsurface samples, however, are difficult and expensive to obtain, so basic information on the numbers, types, and activities of microorganisms is not available for a diverse array of subsurface environments. Thus, the fundamental principles that govern microbial abundance, diversity, interactions, and activity in subsurface environments remain largely unknown (Chapelle 1993; Madsen and Ghiorse 1993; Lovley and Chapelle 1995; Amy and Haldeman 1997; Fredrickson and Fletcher 1999).

Principles describing the influence of surface environmental factors, such as pH, temperature, nutrient availability, and physical structure on abundance, diversity, activity, and function of microbial communities, are transferable, in some cases, to subsurface environments. Many of the bacteria cultured from subsurface environments are similar to well studied bacteria in soil and surface water (Madsen and Ghiorse 1993). Moreover, conditions that encourage or inhibit bacterial growth are likely to exert similar effects in any environment. However, subsurface conditions differ in important ways from surface waters and soils. Microorganisms in surface habitats interact

with a variety of plants, animals, and microfauna that are not generally present in the subsurface. In further contrast to surface environments, subsurface environments have a relative paucity of carbon sources, and the carbon sources that exist naturally have been poorly characterized (Madsen and Ghiorse 1993; Lovley and Chapelle 1995; Amy and Haldeman 1997; Fredrickson and Fletcher 1999). Subsurface environments also undergo fewer and less dramatic temporal variations in, e.g., temperature and carbon supply, than do surface environments. Finally, geochemical conditions in the subsurface are strongly affected by site geology and local groundwater flow regimes. All of these subsurface environmental factors have important effects on contaminant-plume processes.

Three types of approaches are currently employed to study subsurface microbial populations at contaminated sites: traditional culture-based methods, molecular-based methods, and laboratory microcosms. Culture-based approaches are useful for determining the abundance of some physiologic types of organisms in a contaminated environment. Molecular methods may reflect more closely in situ populations, because culture-based results reflect only those organisms that grow on laboratory media (Madsen 1998). Molecular methods include phospholipid fatty acid (PLFA) analyses, which are reviewed by Green and Scow (2000) and nucleic-acid analyses, reviewed by Madsen (2000), both in this issue. PLFA analyses are useful for quantifying active biomass and characterizing community changes over time and space. Nucleic-acid methods can provide information on the identities of the bacteria present and on their catabolic functions. Laboratory microcosm studies using site-derived samples are often used to estimate degradation rates and to enrich specific bacterial populations for further study. Laboratory microcosms can provide important information about microbial processes at a site, if they are carefully monitored to replicate in situ conditions. Application of all of these methods at contaminated sites has provided new and intriguing information on the diversity and function of microbial populations and communities.

All studies of microorganisms in the environment suffer from the fact that fewer than 10% (and possibly fewer than 1%) of the extant bacteria (Pace 1996; Madsen 2000) have been characterized. In a study by Dojka et al. (1998) using DNA-based approaches at a site contaminated by jet fuel and chlorinated solvents, almost a third of the bacteria for which DNA sequences were obtained can be distinguished only by their DNA sequence and belong to groups for which no cultured representative exists. Thus, numerically dominant and as of yet uncultured and therefore unknown populations of microbes exist at this contaminated site, and their role in this contaminated aquifer is certainly unknown. Likewise, when specific genes are not detected in environmental samples, as in several of the studies reviewed below, other unknown genes, possibly coding for similar functions, are likely to be present. The vast majority of catabolic gene sequences that are currently documented encode enzymes common in aerobic bacteria. In contrast, many, if not most, sites contaminated with bacterially degradable organic compounds are anaerobic, as a result of bacterial utilization of oxygen in the presence of the contaminants. Low biomass in subsurface materials presents a continuing challenge for all molecular methods and may bias results from methods in which nucleic-acid sequences are amplified (Tanner et al. 1998).

Evidence indicates that communities of organisms usually work in concert to degrade the organic contaminants at a site. Microbial communities are believed to be similar to communities of plants or animals, in that they are structured largely by interactions among populations and between populations and the abiotic environment. Commonly measured community features, such as the numbers and types of different categories of organisms, or the relative magnitude or rate of a process, such as degradative capacity, are a function of these interactions. However, unlike plants and animals, bacteria are distinguished from each other primarily by their unique metabolic capabilities rather than their structure or appearance. Therefore, understanding bacterial-community composition in terms of the different physiologic groups represented becomes particularly important for understanding biodegradation processes.

Slater and Lovatt (1984) present a classification of seven types of microbial-community interactions in the degradation of organic compounds. Several of their interaction types have been observed in contaminant plumes and are described below. In some cases, these are assemblages in which each member degrades one or more compounds, but each organism can function independently of the others. In other cases, a consortium of bacteria develops, which is defined as a more closely coupled interaction where one member requires the activity of another for substrate metabolism to occur at all. Examples include cases of "combined metabolic attack," where each bacterial partner provides an enzyme required to break down a substrate, and "interspecies product transfer," where one bacterial partner uses a by-product of the first, resulting in a thermodynamic shift that allows degradation of an otherwise recalcitrant substrate. Other interactions described by Slater and Lovatt (1984) are based on cases where one bacterial partner provides specific nutrients, removes inhibitory substances, or modifies growth parameters such as pH for the second, which actually carries out the substrate degradation. The effect of consortium development is that consortia are capable, in concert, of carrying out a metabolic reaction that none of the consortium members could carry out on its own. Slater and Lovatt (1984) conclude that the key to understanding the fate of organic contaminants in the environment is in

understanding community interactions rather than the specific capabilities of individual bacteria.

Bacteria are also distinguished by their ability to exchange genetic material between taxonomic groups more readily than do other organisms (Reanny et al. 1983). Genes that encode enzymes that are needed to degrade contaminants may be transferred from one bacterium to another. In order to degrade some types of contaminants, especially the new types of compounds that humans have created in recent decades, bacteria must acquire or develop new genes to create enzymes with the appropriate functions. Evidence exists for both gene transfer and gene development at contaminated sites, as described below.

One outcome of these two unique aspects of microbial-community structure might be that subsurface microbial processes vary substantially from site to site, based on unique combinations of genes or organisms at those sites. Evidence presented below indicates that some bacterial biodegradative populations may occur at one plume but not at another. If so, substantially more variability in biodegradative processes across sites would occur than could be predicted from site environmental characteristics alone, and identifying and quantifying bacterial populations and their interactions in contaminant plumes would be critical for site assessment.

Adaptation of Microbial Communities Within Plumes

Increases in the numbers of contaminant-degrading bacteria or increases in the rate of degradation of contaminants inside the plume are regarded as one line of evidence of natural bioremediation or the success of a bioaugmentation strategy (National Research Council 1993). Numerous studies have documented increases in the number of contaminant-degrading bacteria inside groundwater contaminant plumes compared to uncontaminated aquifer materials located nearby (reviewed below). This situation is commonly referred to as adaptation of the microbial populations or communities to plume conditions. However, the processes that lead to adaptation of bacteria within a plume are not well understood. Three mechanisms for this process have been proposed: (1) favored growth of a bacterium present at the site prior to contamination; (2) introduction of a contaminant-adapted bacterium from outside the area; and (3) favored growth of a bacterium carrying a unique metabolic pathway that arose in situ by genetic transfer or recombination (van der Meer et al. 1998). Recent use of DNA-based methods has provided some insights into the mechanisms of adaptation. In many cases, a mixed community of microorganisms develops whose combined activities result in effective degradation of the contaminants.

Numbers and Activity of Contaminant Degraders

Many studies have documented increases in microbial numbers in contaminant plumes. At an abandoned refinery near Hünxe, Germany, Zarda et al. (1998) observed greater bacterial and protozoan numbers in aquifer materials highly contaminated with monoaromatic hydrocarbons in contrast to less-contaminated materials. Godsy et al. (1992) report a 100-fold increase in culturable methanogens within the groundwater plume compared to the uncontaminated groundwater at a creosote waste site near Pensacola, Florida, USA, where methanogenic degradation of creosote contaminants is occurring. Pfiffner et al. (1997) describe the groundwater chemistry and the abundances of physiologic groups of bacteria, using culture-based and PLFA approaches, in four locations at a benzene, toluene, ethylbenzene, and xylene (BTEX) -contaminated site near Detroit, Michigan, USA. At this site, highly contaminated sediments had two to four times the bacterial biomass (determined from PLFA) of less-contaminated aquifer materials. Groundwater geochemistry indicates anaerobic conditions in the most contaminated portions of the plume; however, Pfiffner et al. (1997) enumerate only aerobic hydrocarbon-degrading populations. Aerobic hydrocarbon degraders were most numerous in the more aerobic portions of the plume and were not correlated with hydrocarbon concentrations on the sediments.

Other studies document increases in specific contaminant degraders, but non-specific measures of total microbial populations sometimes did not show an increase. Song and Bartha (1990) note an increase in microbial numbers in general, and a specific increase in hydrocarbon degraders in surface and subsurface materials contaminated with jet fuel. Long et al. (1995) compare numbers of toluene- and decane-degrading bacteria in pristine, uncontaminated materials with aquifer materials contaminated with jet fuel or gasoline. They report that the proportion of decane-degrading bacteria was higher in all contaminated samples, and that toluene-degrader numbers were at least an order of magnitude greater in contaminated samples compared to pristine samples. Braddock and McCarthy (1996) investigated a hydrocarbon-contaminated site in the Arctic Ocean where the saturated zone thaws for about 90 days each summer. At this site, the numbers of hydrocarbon-oxidizing microorganisms were 100 times greater in samples from contaminated areas than in uncontaminated samples. In contrast to the study of Song and Bartha (1990), however, populations of total heterotrophs at the site (indicative of a general increase in bacterial populations) do not correlate with contaminant levels.

Some studies show an increase in specific degradative activity within contaminant plumes. At a coal-tar waste site, naphthalene and phenanthrene leaching from disposed coal tar to groundwater were degraded in samples taken from within the plume, but not in

uncontaminated samples (Madsen et al. 1991). In addition, increases in numbers of bacteria and protozoa occurred within the plume. Later studies at the same site (Madsen et al. 1996; Wilson and Madsen 1996) extracted a metabolite of bacterial naphthalene degradation. The presence of the metabolite and the comigration of degradative bacteria with coal-tar-derived aromatic hydrocarbons reflect in situ degradative processes occurring within the plume. Long-term studies of chlorobenzene-degrading bacteria at Kelly Air Force Base in Texas, USA, show that chlorobenzene degraders occur only within the chlorobenzene-contaminated area (van der Meer et al. 1998). Stapleton et al. (1998a) use the abundance of the 16S rDNA gene – see Madsen (2000) for description of the use of this gene – as an indicator of bacterial biomass for a site contaminated with BTEX, and naphthalene at Columbus Air Force Base, Mississippi, USA. Little change in microbial biomass occurred within the plume compared to the uncontaminated background. Activity assays, however, indicate that microorganisms in materials from within the plume are more active than those in materials taken from outside the plume in degrading benzene, toluene, and naphthalene.

In summary, observed increases in biomass, specific contaminant degraders, and degradative activity indicate that subsurface microbial populations adapt to contaminant plumes. It is unclear, however, why at some sites specific assays for degradative microbial populations indicate an increase within the contaminant plume, whereas more general assays (e.g., for bacterial biomass) do not. Possible explanations include (1) changes in activity of already-existing populations without a concomitant increase in their numbers, (2) within-plume variability in populations and subsurface conditions, and (3) artifacts due to the method used. Combined site studies employing interactions between hydrogeologists and microbiologists are most likely to resolve these issues.

Degradative Genes

The nucleic-acid sequences of genes that encode enzymes responsible for critical or unique steps in the degradation of contaminants can be detected in environmental samples (see Madsen 2000). The sequences of some degradative genes have been identified through laboratory studies of selected bacteria known to degrade particular contaminants. Most of the degradative genes identified to date are associated with bacteria that are relatively easy to grow in the laboratory and that degrade common contaminants, such as toluene, naphthalene, and trichloroethylene (TCE). Observations of increases in these characterized degradative genes in contaminant plumes indicate that microbial populations are adapting to exploit the influx of contaminants at a site. Recent results also provide some insights into the mechanism of genetic adaptation.

Hallier-Soulier et al. (1996) examined 12 types of subsurface materials contaminated with fuels or hydrocarbons for the presence of the *xylE* (catechol 2, 3,-dioxygenase) gene, indicative of aromatic hydrocarbon degradation. The *xylE* gene was detected in greater abundance in contaminated than in pristine materials, but the abundance of this gene was poorly correlated with the number of toluene-degrading bacteria present. This result might indicate that toluene-degrading bacteria with other untested or unknown degradation pathways were also present. Guo et al. (1997) report that of five tested degradative genes, the presence of the *xylE* gene is best correlated with fuel-oil contamination at a leaking underground storage site in Washington state, USA. They also detected genes for naphthalene degradation (*nahAcd*) and toluene *para*-monoxygenase (*tmoABCDE*) at lesser levels, but they did not detect genes for alkane hydroxylase (*alkB*) or toluene dioxygenase (*todC1C2BA*).

Stapleton and Sayler (1998) report on the presence of several genes involved in various contaminant degradation pathways at three sites in the USA. These sites are (1) the US Department of Energy Westinghouse Savannah River site in Aiken, South Carolina, contaminated with the chlorinated solvents PCE (tetrachloroethylene) and TCE; (2) Dover Air Force Base, Delaware, at locations contaminated with BTEX, PCE, and TCE; and (3) Columbus Air Force Base, Mississippi, at locations contaminated with BTEX and naphthalene (Stapleton and Sayler 1998). At Savannah River, each gene was present in the population prior to field remedial treatment, and several remedial treatments appeared to decrease some gene frequencies, but results are inconclusive due to low bacterial biomass. Nevertheless, these authors successfully used DNA-detection methods at the other two sites. At Dover Air Force Base, six genes involved in degradation of methane, toluene, naphthalene, and TCE were not detected in uncontaminated materials, but each gene was detected in contaminated areas undergoing natural attenuation. At Columbus Air Force Base, genes for alkane hydrolysis, anaerobic TCE degradation, and aerobic degradation of naphthalene, toluene, and xylene were quantified in an artificially generated plume of benzene, toluene, ethylbenzene, *p*-xylene, and naphthalene undergoing natural attenuation (Stapleton and Sayler 1998). In contrast to the Dover site, each of the degradative genes was also detected in uncontaminated aquifer materials from Columbus Air Force Base. Within the plume, little change in microbial biomass occurred, and the quantity of each gene was similar to that at uncontaminated locations.

Results from the above studies highlight the limitations of current knowledge on degradative genes controlling contaminant biodegradation. At this time, the number of known genes available for analysis is small. Thus, the specific genes for which molecular

approaches are currently available may be present in only part of the degrading population, as in the study by Hallier-Soulier et al. (1996). Presumably, at sites where the use of probes for known degradative genes was unsuccessful, unknown genes exist that contribute to the degradation processes. This may especially apply where the gene probes used are not well matched with site conditions, e.g., where gene probes for aerobic degradation pathways are used to study a site under primarily anaerobic conditions.

Some studies have used gene-based approaches to understand how degradative capabilities are acquired by microbial populations in contaminant plumes. These studies demonstrate that bacteria can either share degradative genes or develop new degradative genes within a specific site. Herrick et al. (1993) analyzed samples for naphthalene degradation using a degradative gene sequence similar to that used by Guo et al. (1997). They analyzed materials from uncontaminated and contaminated locations at a coal-tar waste site and observed the gene at two of four locations within the plume, although naphthalene mineralization was noted at all four locations. They did not detect the gene at an upgradient, uncontaminated site. At the same site, Herrick et al. (1997) report that seven plume-derived bacteria had the same sequence for the naphthalene degradation gene, even though these bacteria belonged to different species. From this evidence, they conclude that the naphthalene degradation gene was transferred recently between bacteria at their study site.

Evidence exists that in some cases degradative genes may originate at a site. Van der Meer et al. (1998) studied the origin of chlorobenzene-degrading bacteria at Kelly Air Force Base, Texas. They conclude that the ability to degrade chlorobenzene, which is rare in aerobic bacteria, was not present initially at the contaminated site but occurs now in a distinctive, site-specific population of aerobic bacteria that have apparently acquired the ability through gene transfer and rearrangement. Chlorobenzene degraders could not be obtained at Kelly Air Force Base from outside the chlorobenzene-contaminated area, suggesting that degrading populations did not exist prior to the introduction of the contaminant. Chlorobenzene-degrading bacteria within the plume at Kelly Air Force Base do not carry the same gene sequences as do other chlorobenzene-degrading bacteria from other sites, suggesting that the gene for chlorobenzene degradation did not arrive with newly introduced bacteria. Instead, some of the genes in the predominant chlorobenzene degrading bacterium at the site are similar to those in other, non-chlorobenzene degraders from the site. This finding suggests that a new chlorobenzene-degradative gene was formed from non-degradative genes in bacteria indigenous to Kelly Air Force Base.

In summary, the usefulness of DNA-based methods is currently limited by the number of known degradative genes. Molecular methods, however, are capable of elucidating the mechanisms by which bacteria at a site acquire degradative capabilities. Further studies identifying new genes and on the capabilities of bacteria to transfer or create genes are needed to fully understand gene abundances and site biodegradative capacities.

Community Structure

Contaminant degradation at a site may not be the result of the activity of a single population of bacteria. Instead, communities composed of multiple bacterial populations, with different degradative capabilities, probably contribute to the overall rate and capacity for degradation measured at a site. Understanding the degree to which degradation is a group as opposed to individual function is important to understanding the potential success of remediation strategies. A particularly interesting example is the degradation of TCE at Moffett Field, California, USA. TCE is a contaminant that cannot be used as an aerobic growth substrate by any known bacteria. Nonetheless, some bacteria produce enzymes that oxidize TCE when they grow on other substrates. This fortuitous reaction is called co-metabolism, or specifically, co-oxidation. Fries et al. (1997) studied populations of phenol- and toluene-degrading bacteria that can co-oxidize TCE following growth on these two primary substrates. They conclude that TCE degradation at Moffett Field, California, is the result of the combined activities of multiple bacterial populations, none of which degraded TCE particularly well. If this is the case, addition of toluene or phenol to stimulate TCE degradation would not necessarily produce the anticipated result. Instead, the addition of phenol or toluene that encouraged the growth of a bacterial population that co-oxidized TCE poorly would result in less TCE degradation than observed with the combined activities of many populations.

Fries et al. (1997) also obtained information about the abundance of common TCE-degradative genes in these same bacteria. They characterized 273 individual phenol- and toluene-degrading bacteria following field tests of phenol- or toluene-mediated TCE degradation at Moffett Field. The population of phenol and toluene degraders was very abundant and heterogeneous in type and level of activity. Only one of five known toluene degradative pathways (toluene *ortho*-monoxygenase, or TOM) was detected by genetic analysis in these 273 bacteria, and 86% of the bacteria that grew on both phenol and toluene carried this gene. Although the TOM pathway of toluene degradation has been implicated in the laboratory as one of the more favorable pathways for TCE co-oxidation, these isolates varied greatly in their ability to co-oxidize TCE, and at best they were moderately effective in comparison to laboratory strains. This result emphasizes the difficulty of relating laboratory results to field conditions. Likewise, Gram-positive bacteria,

which make up about 30% of the 273 bacteria, did not carry any of the known toluene-degradation genes, although they could grow on either toluene or phenol. Therefore, they must possess other, uncharacterized, degradative genes.

Similarly, Ridgway et al. (1990) studied 297 individual gasoline-degrading bacteria from a site contaminated with unleaded gasoline. Most could grow on 2 or 3 of 15 tested gasoline components, and about 75% could grow on toluene. Highly related bacteria (e.g., strains of the same species) often differed in their ability to grow on various gasoline components. These studies indicate that many degradative bacterial populations, varying greatly in their degradative capabilities, may exist at a single site. Understanding how they interact may be important in designing remedial strategies for a site. In particular, the role of competition between microorganisms for resources in contaminant plumes remains virtually unexplored.

Community interactions are also important for understanding the adaptation of populations that use different electron acceptors within contaminant plumes. A particularly well studied microbial consortium requires the coexistence of methanogens and bacteria in the genera *Syntrophus* to perform methanogenic degradation of various contaminants (Ferry and Wolfe 1977; Schink 1997). Using benzoate as a model organic substrate, methanogenic degradation has been shown to be thermodynamically favorable only if methanogens are present to remove H_2 or formate (e.g., Ferry and Wolfe 1977). Thus, a consortium of *Syntrophus* sp. and methanogens together can effect the degradation of benzoate by methanogenesis, although results of laboratory studies indicate that neither organism could carry out this degradative process on its own (Schocke and Schink 1997). Dojka et al. (1998) document coexistence of bacteria similar to the genera *Syntrophus* and *Methanosaeta* in regions of a fuel- and solvent-contaminated aquifer where methane concentrations were typically high. Consortia may be required for other biodegradation reactions, but studies in which no single organism can grow independently on the tested substrate involve unique laboratory challenges. Therefore, the role of microbial consortia in biodegradation reactions is poorly understood.

Communities of Bacteria and Protozoa

Protozoa sometimes occur in contaminant plumes, and recent studies indicate that the bacterial and protozoan populations are correlated. Madsen et al. (1991) note an increase in both bacteria and protozoa in a plume of coal-tar wastes. In contrast, Ludvigsen et al. (1999) observed no protozoa in any samples collected from an anaerobic leachate plume. Zarda et al. (1998) note increases in both bacterial and protozoan numbers in highly contaminated aquifer materials at a hydrocarbon-contaminated site. These investigators used both molecular and culture-based approaches to determine protozoan abundance. As is typically the case for bacteria, more protozoa were detected using the molecular approaches than were detected by culture. Nanoflagellates were the dominant component of the protozoan community. The ratio of protozoa to bacteria remained constant at around $1:10^3$ in this aquifer, suggesting that the protozoan and bacterial populations are closely linked.

Evidence suggests that protozoa are significant in controlling bacterial abundance. Sinclair et al. (1993) observed increases in protozoan numbers in the unsaturated zone and in saturated regions below floating fuel at a site contaminated with aviation gasoline and jet fuel. They relate protozoan abundance primarily to availability of oxygen, and note that protozoa increased along with bacteria in an area undergoing biotreatment with H_2O_2. Despite the increase in bacteria following biotreatment, hydraulic conductivity in the aquifer did not decrease significantly, suggesting that the bacterial clogging of the aquifer pore spaces was reduced by protozoan predation. Similarly, results from modeling of a TCE-contaminated site indicate that predation of bacteria by protozoa might account for population fluctuations in methanotrophs (Travis and Rosenberg 1997).

Protozoa may also be significant in controlling bacterial size by selective predation. A diverse protistan community was observed within groundwater contaminated by a plume of sewage effluent near Cape Cod, Massachusetts, USA (Novarino et al. 1997) that included some species that had been hitherto undescribed (Novarino et al. 1994). Many protozoa (up to 10^5/g) have been observed in both the oxic and suboxic zones of the contaminant plume (Kinner et al. 1997). Results from laboratory studies suggest that the protistan community at Cape Cod may completely consume numbers equivalent to the unattached population of bacteria immediately downgradient from the contaminant source every few days (Kinner et al. 1998). The community of protozoa at Cape Cod is dominated by 2–3 μm nanoflagellates that feed selectively on a class of unattached bacteria that are 0.8–1.5 μm in size (Kinner et al. 1998) and that constitute much of the pore-water biomass within the plume (Harvey and Garabedian 1991).

In summary, relatively few studies have been conducted on the role of protozoa in contaminant plumes. The results of one study indicate that protozoa do not always occur under anaerobic conditions. Other studies indicate, however, that protozoa may be significant under both aerobic and anaerobic conditions in controlling bacterial abundance. There is evidence that selective predation on bacteria in a specific size range may play a role in structuring bacterial communities. Presumably, the role of protozoa in recycling nutrients required for bacterial growth may also be important and needs to be explored.

Influence of Subsurface Conditions

Efforts to delineate site variability that may affect microbial populations or their activities are limited by the ability of investigators to define the hydrogeological environment of contaminant plumes at microbiologically relevant scales. For example, the vast majority of aqueous-chemistry data from contaminated sites is collected from wells for which the open interval spans more than a meter. In contrast, many of the studies reviewed herein demonstrate that changes in site geology, hydrology, and geochemistry relevant to microorganisms can occur on much smaller scales. Studies of subsurface contaminant plumes show that rates of microbial processes determined in situ are sometimes not well replicated by laboratory studies using site-representative materials (Chapelle et al. 1996; Smith et al. 1996). Due to the difficulty of defining site characteristics accurately at microbiologically relevant dimensions, laboratory tests may often fail to reproduce the in situ characteristics significant to the microbial populations carrying out the process of interest.

Several of the studies described above report the inability to detect bacteria or their genes in all the expected locations at a site (e.g., Herrick et al. 1993; Stapleton et al. 1998a). Loeffler et al. (1998) describe variability in the type and extent of degradation of TCE in samples taken from two depths in a shallow, sandy aquifer in Michigan, USA. Nielsen and Christensen (1994) note that aerobic degradation rates of aromatic hydrocarbons varied with location in a hydrocarbon plume. Kao and Borden (1997) describe site-specific variability in BTEX degradation under nitrate-reducing conditions across a survey of four sites in two states. At a site contaminated with jet fuel and solvents, Dojka et al. (1998) document that zones geochemically defined as iron-reducing or sulfate-reducing did not harbor significant populations of currently known iron- or sulfate-reducing bacteria. Anderson and Lovley (1999) note the occurrence of spatial variability in naphthalene and benzene biodegradation at a petroleum-contaminated site in Bemidji, Minnesota, USA.

These results suggest that a better understanding is required of the hydrologic and geochemical conditions controlling microbial habitats in the subsurface. Recent studies document systematic variations over time and space in microbial populations in contaminant plumes. Using interdisciplinary approaches, the population variations have been related to changes in subsurface geochemical and hydrologic conditions. Thus, encouraging evidence is emerging that known principles of microbial adaptation to surface habitats can be applied to the subsurface once the hydrologic and geologic controls on geochemical conditions and nutrient supply are better understood. *Figure 1* illustrates some currently understood factors that influence subsurface habitats in contaminant plumes. The importance of each depicted influence is documented in various studies that are summarized below.

Hydrology and Geology

Understanding site hydrology is critical to evaluation of contaminant-plume development. Nevertheless, very few studies document the effect of hydrologic parameters on microbial populations in contaminant plumes. Several studies have examined the development of terminal-electron-accepting-process (TEAP) zones, where variations in microbial physiological processes occur along groundwater flow paths and with depth in contaminant plumes (Chapelle 1993; Chapelle et al. 1995; Smith 1997). Characterization of physiological processes is important because the degradation potential of specific compounds varies depending on the TEAP. Typically, TEAP zones are delineated by measuring, for example, concentrations of electron donors and acceptors in pore-water samples from wells. However, interpretation of these data is problematic, because concentrations of these constituents are affected not only by microbial reactions but also by advective transport, dispersion, and inorganic reactions. In addition, because geochemical gradients exist in plumes on vertical scales of less than 1 m, pore-water samples from wells with large screen lengths, which are typical at many contaminated sites, may be sampling waters from two or more TEAP zones. By sampling aquifers at small spatial scales, several studies illuminate the underlying processes

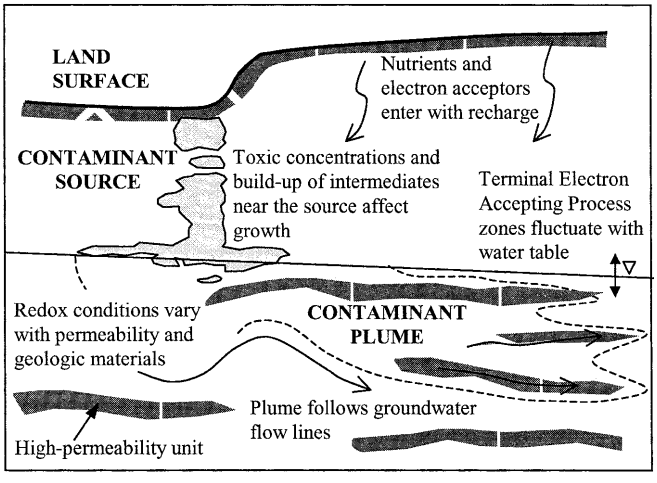

Figure 1 Conceptual illustration of how subsurface environmental conditions influence microbial populations in contaminant plumes. Near contaminant source, some members of the microbial community may be inhibited by toxic concentrations of the contaminant, thereby leading to decreased growth and buildup of intermediate compounds. In vicinity of capillary fringe and water table, an influx of nutrients and electron acceptors with groundwater recharge may result in greater growth. Redox conditions in this area may vary with seasonal water-table fluctuations. Below water table, contaminant plume and associated redox zones follow curves of groundwater flow lines. Redox conditions often vary among zones of high and low permeability

controlling the zonation of microbial physiologic types that correspond to various TEAPs.

Significant vertical and horizontal variations in TEAP zonation and associated degradation processes have been noted in a variety of settings (Christensen et al. 1994; Norris et al. 1994). Smith et al. (1991a) document the requirement for closely spaced vertical sampling intervals to delineate microbiological TEAP and degradative gradients at contaminated sites. At the Borden aquifer in Canada, Rajaram and Gelhar (1991) examined vertical dispersivity in the field, and they report values that are on the order of molecular diffusion. Similarly, at a sewage-effluent plume near Cape Cod, Massachusetts, studies with large-scale tracer tests estimate the vertical dispersivity in the aquifer to be 0.15 cm, or >600 times smaller than longitudinal dispersivity (Garabedian et al. 1991; LeBlanc et al. 1991). The small vertical dispersivity at the Cape Cod site limits vertical mixing and maintains sharp gradients in oxygen and other solutes, even after several kilometers and several decades of transport (Smith et al. 1991a). Concomitant with the oxygen gradients are large changes over a vertical distance of a few meters in bacterial abundance, growth rates, and heterotrophic activity, as well as cell morphology (Smith et al. 1991b). In the horizontal direction, the plume character at Cape Cod changes more gradually downgradient over a scale of kilometers. An increasing recalcitrance of the residual dissolved organic carbon (DOC) occurs (Harvey and Barber 1992), and some studies report an increasing importance of autotrophic processes (such as nitrification) as an oxygen-consuming mechanism (Ceazan 1987; Ceazan et al. 1988).

At a crude-oil spill site near Bemidji, Minnesota, Essaid et al. (1995) note variations in hydrologic flow paths in response to restricted recharge through the non-aqueous oil body. Variations in flow paths resulted in dramatic changes in the overall depth of the anaerobic portion of the plume, where ferric iron reduction and methanogenesis were the dominant TEAPs. At the same site, Bekins et al. (1999) show that areas where methanogenic conditions had developed are clearly indicated by shifts in the numbers and types of culturable organisms attached to the materials. In the methanogenic intervals, these workers report increased numbers of methanogens and heterotrophic fermenters and fewer iron reducers. Even though methane concentrations were near saturation throughout the anoxic zone, which is 2–4 m in vertical thickness (Cozzarelli et al. 1996), culturable methanogens exist in narrow intervals spanning only 25–50% of this zone.

Ludvigsen et al. (1998, 1999) studied microbial populations and redox processes in 37 samples taken from an aquifer contaminated by landfill-leachate near Grindsted, Denmark. As in other studies, this research demonstrates that landfill leachate modifies aquifer conditions to support enhanced growth of specific populations of bacteria and fungi. Numbers of all bacteria (by direct count) and biomass (by PLFA) were highest in the most contaminated portion of the aquifer, near the landfill margin. In this same area, methane production and sulfate reduction were observed, and numbers of methanogens and sulfate reducers (determined by most probable number, MPN) were greatest. Downgradient of the landfill margins, sulfate reducers could still be detected, as well as iron, manganese, and nitrate reducers. PLFA profiles also indicate that a shift in community composition occurred along the downgradient transect, and some specific PLFAs support the interpretation of increased abundance of sulfate reducers near the landfill border. However, no clear relationship was observed between PLFA biomarkers for specific redox-related populations and quantitative measures of redox processes in the same samples. Most samples exhibited more than one co-occurring redox activity, although usually one process dominated. This work substantiates the need to understand the structure of both communities and populations in contaminant plumes in order to interpret degradation processes.

Fluctuations in recharge to shallow contaminant plumes can create temporal variability in geochemical conditions that is reflected in microbial population changes. Vroblesky and Chapelle (1994) describe changes in site TEAP zonation in response to the influx of sulfate with recharge. McGuire et al. (1999) document significant temporal variation, on the scale of months, in TEAP zonation in a groundwater plume containing fuel and chlorinated solvents near Oscoda, Michigan, USA. Concomitant changes were noted in results of DNA-based analyses of microbial-community structure at this site over similar temporal intervals (Haack and Reynolds 1999). At the same site, methanogen populations were studied using RNA extracted from active bacteria in the aquifer materials (Reynolds and Haack 1999). Results suggest that populations of methanogens (or other *Archaea*) were most abundant in materials where the data indicate that methanogenesis or sulfate reduction is the predominant TEAP during most of the year. Dramatic changes in methanogen (or other *Archaea*) abundance occurred over vertical intervals of less than 1 m on each tested date.

An understanding of site geology, hydrology, and hydrochemistry is required to avoid misinterpreting TEAP zonation. Yager et al. (1997) carefully delineate the hydrogeologic setting of a fractured dolomite aquifer contaminated with chlorinated ethenes. Although methane and sulfide analyses in some wells suggest that methanogenesis or sulfate reduction is a possible TEAP, the authors were able to discount both degradative processes at the site, on the basis of hydrogen-gas analysis and by recognizing that the source of the methane and sulfide is from a deeper and non-contaminated aquifer unit. Background hydrochemistry also affects the availability of various

electron donors and acceptors. For example, sulfate reduction may not be important in certain plumes because sulfate is not available in site pore waters (Baedecker et al. 1993; Bekins et al. 1999). Yager et al. (1997) conclude that reductive dechlorination of TCE is accelerated by addition of pulverized dolomite from one major geologic unit at the site. These authors hypothesize that electron donors in the dolomite (possibly carbon-rich bitumen) are responsible for this result.

When ferric iron minerals are present at a site, bacterially catalyzed oxidation of aromatic hydrocarbons may be coupled to ferric-iron reduction (Lovley et al. 1989). The presence and depletion of ferric iron may also affect the development of other TEAP zones within a contaminant plume. At the crude-oil contaminated site near Bemidji, Minnesota, Baedecker et al. (1993) report that degradation of the hydrocarbons by iron reduction initially dominated. However, as ferric iron was depleted (Tuccillo et al. 1999), narrow methanogenic zones formed in areas of high contaminant flux (Bekins et al. 1999). Degradation of benzene (Anderson et al. 1998; Rooney-Varga et al. 1999) and naphthalene (Anderson and Lovley 1999) occurred at the Bemidji site by ferric-iron reduction only in locations where ferric iron was not depleted, and such sites tended to be in downgradient locations, away from methanogenic zones. At these locations, the rate of anaerobic benzene or naphthalene degradation is related to the percentage of ferric iron in the aquifer materials (Anderson and Lovley 1999). These active locations have higher abundances of bacteria in the Geobacteraceae family (a ferric iron-reducing group) but do not contain overall abundances of ferric-iron-reducing bacteria that are different from those at other site locations.

Albrechtsen et al. (1995) observe that sediment samples taken from near a landfill had low concentrations of ferric iron, whereas those collected at more downgradient locations had higher concentrations. Negligible ferric-iron reduction was observed in the ferric-iron-depleted samples under in situ conditions, although the same samples amended with ferric iron and acetate carried out ferric-iron reduction. Likewise, submeter-scale intervals of sulfate reduction at a gasoline-contaminated site near Galloway, New Jersey, USA, are attributed to local depletion of microbially reducible solid iron-oxyhydroxides (Cozzarelli et al. 1999).

Variability in the physical structure of aquifer materials influences bacterial transport (Harvey et al. 1993). Lawrence and Hendry (1996) review the role of geologic, hydrologic, and solute variables on bacterial transport through geologic media. Their review indicates that variations in site hydrogeology may significantly influence the location and structure of subsurface microbial communities by differential transport of bacteria. Aelion (1996) notes increased contamination and decreased biodegradation of jet fuel in clay-dominated samples relative to more permeable materials, taken from closely spaced vertical intervals at a single site. Ulrich et al. (1998), using micro-scale radiography, note decreased sulfate-reducing activity in clay layers of unconsolidated contaminated materials, as compared to sand layers. Ludvigsen et al. (1999) report that PFLA analyses reveal increased populations and changes in community structure in clay and silt lenses compared to sands in an aquifer contaminated by a landfill-leachate plume. These observations suggest that significant small-scale heterogeneity in biodegradation may be expected, simply due to heterogeneity in aquifer materials.

Temperature

Various bacterial genera are adapted to temperature extremes (Brock and Madigan 1989). Therefore, biodegradation might take place in especially cold or hot environments. Indeed, biodegradation of contaminants at low temperatures is documented (Atlas 1986; Braddock and McCarthy 1996). Conversion of toluene to carbon dioxide occurred at similar rates in materials from Alaska incubated at 5 °C and from South Carolina incubated at 20 °C (Bradley and Chapelle 1995). No similar study has been conducted at a high-temperature site, although various thermophilic bacteria capable of carrying out fermentative metabolism, sulfate reduction, or sulfide oxidation have been isolated from moderately high-temperature oil-field production waters (Stetter et al. 1993; L'Haridon et al. 1995).

pH

Acidic or alkaline conditions are relatively common in both pristine (Hem 1989) and contaminated groundwater. A pH of less than 6.5 exists at sites where degradation of creosote (Godsy et al. 1992), crude oil (Baedecker et al. 1993), or jet fuel (Chapelle et al. 1996) has occurred. Although a circumneutral pH is commonly assumed to be the optimum for microbial biodegradative activity (Norris et al. 1994), few studies have been conducted on the effects of pH on intrinsic biodegradation. Stapleton et al. (1998b) report that naphthalene and toluene were degraded in effluents from a long-term coal pile storage basin that exhibits pH values as low as 2.0. They suggest that either the microbial community possesses unique degradative pathways not represented in commonly studied bacteria, or a consortium of microorganisms is required to carry out the degradation in this acidic environment. They note that common catabolic genes associated with the degradation of naphthalene and toluene in bacteria that require neutral pH could not be detected by DNA-based methods in samples. Instead, an undefined consortium of several bacteria, a fungus, and a yeast from the site did metabolize 27% of the supplied naphthalene in 1 week in laboratory tests.

Nutrient Availability

Many common organic contaminants (aromatic and chlorinated aliphatic hydrocarbons) are composed primarily of carbon and hydrogen. Therefore, nitrogen and phosphorus limitations are likely if the contaminant can be used as a carbon source by indigenous microbial populations (Ghiorse and Wilson 1988). At the crude-oil spill site in Minnesota, Bekins et al. (1999) observed that culturable methanogens are 100 times higher in the contaminated unsaturated zone than in the plume below the water table. These results suggest that access to nutrients entering with recharge water is promoting microbial growth in the unsaturated zone. This finding is consistent with results from this site of Rogers et al. (1998), who observed that phosphate-bearing minerals placed in contaminated wells were dissolved and concluded that the microbial population was phosphate-limited. Franzmann et al. (1996), using PLFA profiles at a BTEX-contaminated site in Perth, Australia, report that levels of biomass increased with proximity to the root zone, suggesting that plants are a source of nutrients in the subsurface.

Nutrient amendment to enhance hydrocarbon degradation in marine oil spills and contaminated soils is relatively common; however, the difficulties of delivering nutrients to the subsurface have prevented similar tests for most groundwater plumes. Fuller et al. (1995) demonstrate that the rate of TCE and toluene degradation in vadose-zone materials from a gasoline-contaminated site was limited by the availability of mineral nutrients. In contrast, Smith and Duff (1988) demonstrate that denitrification of nitrate, a primary contaminant in a sewage-effluent plume at Cape Cod, was electron-donor (carbon) limited in a zone 5–7 m thick that extends for several kilometers downgradient from the contaminant source. N and P were added to a plume of TCE undergoing aerobic degradation by methanotrophic bacteria at the Savannah River, South Carolina, site; this addition, however, had little effect on methanotroph numbers in the plume (Travis and Rosenberg 1997). These few results are not enough to suggest the general availability of nutrients in the subsurface, nor their effect on degradative processes.

Contaminant Toxicity

Contaminants may exert toxic effects on some bacteria or bacterial metabolic pathways. Many contaminant plumes contain mixtures of known organic contaminants together with other poorly characterized organic compounds, and, in some cases, heavy metals or other potentially toxic inorganic compounds. Exposure to various combinations of these contaminants is likely to have significant effects on microbial-population structure from one plume to another, but these effects remain poorly described. For example, toxic effects on hydrocarbon degradation have been noted for organosulfur compounds (Londry and Suflita 1998). In addition, landfill leachate toxicity is hypothesized by Ludvigsen et al. (1999) to explain low concentrations of PFLAs and reduced rates of methanogenesis and sulfate reduction in the areas of highest leachate concentrations. The toxic effect of heavy metals on microbes is extensively documented (e.g., Collins and Stozky 1989), and heavy metals are known to decrease degradation rates of organic contaminants (e.g., Kuo and Genthner 1996). A better understanding, however, is needed of the in situ effects of heavy metals on degradation of organic contaminants.

Hydrocarbons can also be toxic to bacteria (Sikkema et al. 1995). Although Mormile and Suflita (1996) indicate that no inhibition of methanogenesis occurred by selected gasoline hydrocarbons, other studies document significant hydrocarbon toxicity effects. Guo et al. (1997) report that the detection of the *xylE* gene increased with aromatic hydrocarbon concentrations on materials of up to 100 mg/kg, but detection decreased at higher concentrations, perhaps due to toxicity. Similarly, decreased populations in response to increased gasoline concentrations are noted by Long et al. (1995). Fries et al. (1997) document sensitivity of toluene-degrading bacterial isolates from contaminated plumes to the concentration of toluene, and Song and Bartha (1990) conclude that enzymatic activity is inhibited by some jet-fuel components. Likewise, Warren et al. (1999) note toxicity of crude oil to acetate-utilizing methanogens. Within the crude-oil plume, acetate-utilizing methanogens were detected only at locations distant from the non-aqueous oil body. In laboratory microcosms, gas production from added acetate was inhibited by the presence of crude oil. Laboratory studies of acetate-utilizing methanogens from a creosote-waste-contaminated site indicate that they also are inhibited by the soluble fraction of the creosote compounds (Bekins et al. 1997).

Summary and Conclusions

Studies of contaminant plumes show that subsurface microbial populations adapt to contaminant plumes in several ways. At all studied sites, either the activity or the number of specific degraders has increased. In most cases, non-specific measures of microbial numbers also indicate an increase. Studies of increases in degradative genes within plumes using molecular methods are beginning to yield insights into the mechanisms of adaptation. Evidence exists that new contaminant-degrading genes unique to a site may be formed from previously existing non-degradative genes. In other cases, evidence for transfer of a contaminant-degradation gene between species has been documented. The results of many studies, however, are less conclusive. Two important difficulties may explain some of the inconclusive results. First, the use of molecular methods is limited to genes that either

are known or can be isolated from the environment. However, ample evidence indicates that most degradative genes, particularly those for anaerobic degradation, are still unknown. Second, many studies indicate strong spatial variability in physiologic type and genetic capabilities. These results suggest that a better understanding is needed of how spatial variation in the subsurface environment results in microbial variability.

Evidence shows that degradation reactions are carried out by interacting communities of microorganisms with different capabilities working in concert. For example, many types of bacteria capable of degrading a single compound may be present at a site, but they may differ in degradative genes used, degradation rates, or growth characteristics. Moreover, highly related bacteria may grow on different compounds of a complex mixture of organics such as gasoline. Presumably, this specialization reduces competition for substrates in contaminant plumes. The necessity for coordinated action of a consortium in methanogenic degradation of organics has been well documented in the laboratory, and recently known members of a methanogenic consortium were observed in a fuel- and solvent-contaminated aquifer. The role of protozoan predation in structuring microbial populations is important at some sites.

Numerous features of subsurface environments exert significant influence on microbial community structure and on the processes that these communities carry out under pristine or contaminated conditions. These features include pore-water chemistry, permeability, grain size, mineralogy, recharge characteristics, temperature, and susceptibility to seasonal variations in factors such as temperature and recharge. Features such as these might be used to categorize subsurface environments, in much the same way that lakes, streams, or soils can be grouped by physical and chemical features that influence microbial activity. This review highlights promising results on the numbers, types, and activities of bacteria in contaminant plumes. As more studies become available from culture-based and molecular approaches, a synthesis of very practical information for the remediation community will evolve.

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