

Bioremediation of Petroleum Hydrocarbon-Contaminated Ground Water: The Perspectives of History and Hydrology

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Abstract

Bioremediation, the use of microbial degradation processes to detoxify environmental contamination, was first applied to petroleum hydrocarbon-contaminated ground water systems in the early 1970s. Since that time, these technologies have evolved in some ways that were clearly anticipated by early investigators, and in other ways that were not foreseen. The expectation that adding oxidants and nutrients to contaminated aquifers would enhance biodegradation, for example, has been born out by subsequent experience. Many of the technologies now in common use such as air sparging, hydrogen peroxide addition, nitrate addition, and bioslurping, are conceptually similar to the first bioremediation systems put into operation. More unexpected, however, were the considerable technical problems associated with delivering oxidants and nutrients to heterogeneous ground water systems. Experience has shown that the success of engineered bioremediation systems depends largely on how effectively directions and rates of ground water flow can be controlled, and thus how efficiently oxidants and nutrients can be delivered to contaminated aquifer sediments.

The early expectation that injecting laboratory-selected or genetically engineered cultures of hydrocarbon-degrading bacteria into aquifers would be a useful bioremediation technology has not been born out by subsequent experience. Rather, it appears that petroleum hydrocarbon-degrading bacteria are ubiquitous in ground water systems and that bacterial addition is usually unnecessary. Perhaps the technology that was least anticipated by early investigators was the development of intrinsic bioremediation. Experience has shown that natural attenuation mechanisms — biodegradation, dilution, and sorption — limit the migration of contaminants to some degree in all ground water systems. Intrinsic bioremediation is the deliberate use of natural attenuation processes to treat contaminated ground water to specified concentration levels at predetermined points in the aquifer. In current practice, intrinsic bioremediation of petroleum hydrocarbons requires a systematic assessment to show that ambient natural attenuation mechanisms are efficient enough to meet regulatory requirements and a monitoring program to verify that performance requirements are met in the future.

Introduction

In the summer of 1971, a gasoline pipeline in Whitemarsh Township, Pennsylvania, broke and released several hundred thousand gallons of high-octane gasoline. Over the next year, an estimated 100,000 gallons (379,000 L) of gasoline seeped to the underlying dolomitic aquifer and dissolved petroleum hydrocarbons migrated to a nearby municipal water supply well. At that time, it had only recently been proposed that ground water systems might contain microorganisms capable of degrading petroleum hydrocarbons (Litchfield and Clark 1973). Furthermore, it was becoming clear that physical methods for removing gasoline from aquifers were not particularly efficient (McKee et al. 1972). A logical approach to this problem, therefore, was to consider using indigenous hydrocarbon-degrading microorganisms to clean up the contaminated aquifer. Accordingly, a group of microbiologists work-

ing in the oil industry documented the presence of petroleum hydrocarbon-degrading bacteria in the ground water of the Whitemarsh site, and showed that the growth of these microorganisms could be stimulated in the laboratory by providing oxygen, nitrogen, and phosphate (Jamison et al. 1975). Furthermore, they showed that this same effect could be produced under field conditions by adding nutrients and air-saturated water to contaminated wells. These authors concluded that, if nutrients and oxygen could be efficiently delivered to ground water, then "the removal of gasoline might be accomplished in a matter of months rather than the years it would take to physically remove it." This was one of the first instances where bioremediation was actively considered as a remedial strategy to clean up a petroleum hydrocarbon-contaminated ground water system.

Since these beginnings in the early 1970s, bioremediation of petroleum hydrocarbons has become an accepted technology and has grown into an industry making more than \$290 million a year in the United States alone (Glass et al. 1995). This paper reviews the history of these technologies specifically as they have been applied to ground water systems. This history shows that the current practice of bioremediation includes many concepts and methods developed at the very beginning of the technology. Conversely,

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some approaches that were thought to be quite promising in the early 1970s have not come into common use. This history also shows that some of the most useful current practices (intrinsic bioremediation, for example) were not anticipated by early investigators in the 1970s.

As bioremediation technology for ground water systems developed, there has been a gradual realization that hydrologic considerations are extremely important. Ground water systems are inherently inaccessible, usually heterogeneous, and difficult to accurately characterize. Experience has shown that favorable hydrologic conditions, and a clear understanding of these conditions, are critical to the success of bioremediation. When this understanding can be properly exploited, either by controlling and directing ground water movement in engineered bioremediation systems, or by accurately assessing ambient rates and directions of ground water flow in intrinsic bioremediation systems, a reasonable degree of success is possible. The literature clearly documents these hard-earned lessons. As such, this literature is a valuable resource that may facilitate the development of bioremediation strategies for chemical contaminants other than petroleum hydrocarbons.

Marine Oil Spills and the Biodegradation of Petroleum Hydrocarbons

The early 1970s were a time of intense scientific interest in the biodegradation of petroleum hydrocarbons (Atlas and Bartha 1973). This interest stemmed largely from environmental concerns surrounding large-scale oil spills in the ocean, such as the sinking of the supertanker Torrey Canyon in the English Channel in 1967 (Atlas 1981). At that time, it was widely feared that contamination from such spills could result in permanent and irreversible damage to the environment. Although it had been known since the 1940s that petroleum hydrocarbons were degraded by microorganisms (Zobell 1946), the extent to which these processes occurred and the conditions that limited or enhanced biodegradation in aquatic environments were a matter of vigorous inquiry (Atlas and Bartha 1972; Floodgate 1972; Bartha and Atlas 1977).

These studies were instrumental in identifying important metabolic pathways by which petroleum hydrocarbons were degraded. In addition, these studies provided a framework by which the relative biodegradability of different compounds could be understood (McKenna and Kallio 1964; Gibson 1971; Pirnik 1977). It was found, for example, that *n*-alkanes were generally more biodegradable than branched alkanes, alkenes, or cyclic compounds present in petroleum (Treccani 1964). Degradation of *n*-alkanes proceeded via oxidation of a terminal hydrocarbon forming a long-chain carboxylic acid (Ratledge 1978). It was also found that methyl branches on aliphatic hydrocarbons tended to increase resistance to microbial attack (Pirnik 1977). The biodegradation of aromatic compounds was found to involve an oxidative attack upon the ring structure, the formation of a diol intermediate, followed by ring cleavage and the formation of organic acids (Gibson 1971). For aromatic compounds, simple methyl substitutions on the benzene ring — such as in toluene or various xylene isomers — were found to enhance biodegradability (Gibson and Subramanian 1984).

These early studies also emphasized the importance of oxygen and trace nutrients for the biodegradation of petroleum hydrocarbons. In particular, it was widely observed that hydrocarbon biodegradation was limited by available nutrients such as nitrogen and phosphorus (Atlas and Bartha 1972; Floodgate 1972).

Accordingly, it was reported that addition of such nutrients could significantly enhance rates of biodegradation (Bridie and Bos 1971). These studies also suggested that oxygen was the only terminal electron acceptor that supported significant rates of petroleum biodegradation (Bailey et al. 1973). These findings greatly influenced early bioremediation strategies in ground water systems, where an emphasis was placed on nitrogen, phosphate, and oxygen addition to enhance biodegradation (Jamison et al. 1975).

Studies of oil spills in the marine environment also led to the early development of genetic engineering techniques for enhancing biodegradation. Most notable in this context were the studies of A.M. Chakrabarty and his coworkers (Chakrabarty et al. 1973). These investigators knew that certain strains of *Pseudomonas* had the capability for degrading particular aliphatic hydrocarbons. One strain of *Pseudomonas aeruginosa*, designated strain AC59, was capable of degrading C₆, C₈, and C₁₀ compounds, but could not degrade longer aliphatic compounds. Another strain, designated strain AC63, could degrade C₁₂ and C₁₄ molecules, but could not degrade shorter hydrocarbons. Because these degradative capabilities were plasmid-encoded, it was possible to combine them into a single organism using the techniques of genetic engineering. This new microorganism was capable of degrading C₆ through C₁₈ hydrocarbons. In the landmark case *Diamond v. Chakrabarty*, the U.S. Supreme Court ruled that such genetically engineered petroleum hydrocarbon-degrading microorganisms constituted a manufactured product, and qualified for patent protection. In the early 1970s, and largely because of the work of Chakrabarty and others, it was widely believed that engineered microorganisms would be the basis of many bioremediation strategies. However, fears surrounding the release of genetically engineered microorganisms into the environment have largely been responsible for preventing such strategies from being applied in the field (Forsyth et al. 1995).

Microbial Degradation Processes in Shallow Water Table Aquifers

By the early 1980s, it was apparent that the contamination of shallow water table aquifers by petroleum hydrocarbons, largely due to leaking underground storage tanks and their transfer lines, was a significant environmental problem (Council on Environmental Quality 1981). However, methods for remediating such contamination events were problematic. By far, the most commonly employed remediation strategy was to pump contaminated ground water out of the ground and treat it to remove contaminants. Many of these pump-and-treat systems, as they came to be called, did not consider the possibility that microbial degradation processes occurred (Yazicigil and Sendlein 1981). The reason for this was that, in the early 1980s, little was known about the microbiology of shallow water table aquifers (Wilson et al. 1983). There had been a long tradition, beginning in the 1920s, of studying microbial processes in deep petroleum reservoirs (Bastin 1926). This tradition was carried on by Zobell and his associates throughout the 1930s and 1940s (Davis 1967). But with few exceptions (Litchfield and Clark 1973), there were virtually no investigations of the shallow water table aquifers that were most commonly contaminated by leaking gasoline storage tanks.

Early studies of soil microbiology indicated that numbers of bacteria dropped off sharply with depth (Waksman 1916), and in the early 1980s it was widely assumed that shallow aquifers were largely sterile. With the increasing concern about petroleum hydro-

carbon contamination providing much of the impetus, however, the microbiology of shallow aquifers became an active topic of investigation in the 1980s (White et al. 1983; Hirsch and Rades-Rohkohl 1983). Several of these studies focused on the ability of subsurface microorganisms to degrade petroleum hydrocarbons (Ehrlich et al. 1985). In particular, Wilson et al. (1983) showed that the potential for microorganisms to degrade toluene, a common component of gasoline, was apparently widespread in shallow aquifers.

Throughout the 1980s, laboratory studies of microbial activity in shallow aquifer systems, and the ability of those microorganisms to degrade petroleum hydrocarbons, was a topic of intense inquiry (B.H. Wilson et al. 1986; Heitkamp and Cerniglia 1988; Fredrickson et al. 1991). In addition, several field studies showed that microbial oxidation of petroleum hydrocarbons had significant effects on the chemistry of ground water (Cozzarelli et al. 1989; Chiang et al. 1989) and on the chemistry of aquifer solids (Bennett and Siegel 1987; Baedecker et al. 1992). These field studies also suggested that petroleum hydrocarbons were being oxidized under anaerobic conditions (Cozzarelli et al. 1990). This was unexpected. Throughout most of the 1970s, it was widely believed that molecular oxygen was necessary to biodegrade petroleum hydrocarbons (Bailey et al. 1973). In fact, the first microorganism capable of completely mineralizing an alkyl benzene under strictly anaerobic conditions was only first isolated in 1988 (Lovley and Phillips 1988). Laboratory studies have now shown that petroleum hydrocarbons are degraded under anaerobic conditions (Hunt et al. 1995), including nitrate-reducing (Hutchins et al. 1991a), Fe(III)-reducing (Lovley et al. 1989), sulfate-reducing (Edwards et al. 1992), and methanogenic (Grbić Galić and Vogel 1987) conditions. For many years, the anaerobic oxidation of petroleum hydrocarbons was controversial, largely because aerobic microbial processes had been the focus of the early studies of the marine environment. Nevertheless, the importance of anaerobic oxidation processes in the biodegradation of petroleum hydrocarbons is now firmly established (Lovley 1997).

These laboratory and field studies provided a basis for the design and operation of engineered bioremediation systems for petroleum hydrocarbon-contaminated ground water (Hutchins et al. 1991b). In this regard, these studies were important and successful. However, because the laboratory studies were carried out under controlled (and therefore artificial) conditions, they could not anticipate the difficulties that would be encountered when attempting to deliver nutrients and electron acceptors to ground water systems. These difficulties would only become fully evident during field-scale testing of bioremediation systems.

Engineered Bioremediation of Petroleum Hydrocarbons in Ground Water

The engineered bioremediation systems for treating petroleum hydrocarbon-contaminated ground water, first conceived in the early 1970s, were based largely on observations made from marine petroleum spills. The growth and metabolism of microorganisms in natural environments is always limited by the availability of electron donors, electron acceptors, or other essential nutrients (Atlas 1981). In petroleum spills, there is no lack of electron donors (petroleum hydrocarbons), so microbial metabolism is generally limited by the availability of electron acceptors (such as oxygen, nitrate, sulfate, or Fe(III)), or by the availability of essential nutri-

ents (such as nitrogen, potassium, and phosphate). The basic principle of engineered bioremediation systems is to relieve the lack of electron acceptors and nutrients and thereby increase rates of petroleum hydrocarbon degradation.

In 1974, a patent was issued to Richard Raymond of Suntech Oil (Raymond 1974) for a method that added nutrients and an electron acceptor (oxygen) to aquifers in order to stimulate petroleum hydrocarbon biodegradation. This patent, entitled "Reclamation of Hydrocarbon Contaminated Ground Waters," describes how nutrients and oxygen can be introduced to aquifers by injection wells, and the water can be circulated through contaminated aquifer materials by pumping wells. Many engineered field-scale systems that have been built and tested in the United States are variations on the concept originally patented by Suntech Oil.

Many of the early case studies that used the Suntech Oil process were not described in the peer-reviewed scientific literature, although it was reported that the process enjoyed "reasonable" success in several instances (J.T. Wilson et al. 1986). One of the first case studies reported in the peer-reviewed literature using this basic methodology was described by Wilson and Brown (1989). In this case, approximately 900 gallons (3411 L) of leaded gasoline had leaked into a fractured dolomite overlain by silty sands and gravels and was impacting a nearby municipal well. The first part of the remediation effort was designed to collect free product using a dual pump extraction system that recovered about 100 gallons (379 L) of free-phase gasoline. After a laboratory pilot study showed that addition of ammonium, phosphate, and hydrogen peroxide (to serve as a source of oxygen) could stimulate microbial degradation of hydrocarbons, a full-scale field system was constructed. The nutrients were added through injection wells and an infiltration gallery so that soils above the water table would also be treated, and a pumping well was used to separate free-phase fuel and to draw nutrients toward the zone of contamination. After 18 months of system operation, the zone of contamination was reported to have decreased in size by a factor of 10, with concentrations of total petroleum hydrocarbons reduced below one part per billion.

Most early applications of the Suntech process did not clearly distinguish between degrading or removing free-phase petroleum products, sorbed or insoluble aliphatic hydrocarbons, or soluble benzene, toluene, ethylbenzene, and xylene (BTEX) compounds. Jamison et al. (1975) explicitly state that gasoline is a complex mixture of hydrocarbons, and focus on the biodegradability of relatively insoluble compounds such as *n*-pentane and octane. However, the most common way of judging the effectiveness of engineered bioremediation was by measuring soluble hydrocarbon compounds in ground water (Wilson and Brown 1989). It is not always clear, therefore, whether the engineered bioremediation was designed to remove soluble BTEX from ground water or to remove free-phase or sorbed fuel from aquifer sediments. Some studies combined monitoring of BTEX in ground water with monitoring total petroleum hydrocarbons (TPH) or free-phase fuel in aquifer sediments in order to evaluate the effectiveness of engineered bioremediation (Hutchins et al. 1991b; Wilson et al. 1994a). These studies indicated that removal of soluble BTEX was more efficient than removal of insoluble hydrocarbons.

The use of hydrogen peroxide as a source of oxygen was commonly applied in many early bioremediation systems. Molecular oxygen is only slightly soluble in water (~8.0 mg/L for water in contact with air at 20°C), so oxygen delivery from air-saturated water from injection wells or infiltration galleries is inher-

ently limited. Hydrogen peroxide (H_2O_2), in contrast, is completely miscible in water and will spontaneously decompose giving a source of oxygen ($2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$). Adding hydrogen peroxide to infiltrating water was hoped to greatly increase the delivery of oxygen to contaminated aquifer sediments.

One hydrogen peroxide-based bioremediation system, designed to remediate an aviation gasoline-contaminated aquifer in Traverse City, Michigan, was described by Wilson et al. (1994a). This study was designed, in part, to compare the results of oxygen addition by ground water aeration with oxygen enhancement from hydrogen peroxide. For both oxygen delivery methods, ground water was also amended with ammonium chloride, disodium phosphate, and potassium phosphate to provide essential nutrients. For the first 90 days of operation, oxygen was delivered solely by aeration. In this time, concentrations of oxygen 31 feet (9.45 m) downgradient of the infiltration wells did not increase and BTEX concentrations were not observed to decrease. After the addition of hydrogen peroxide, dissolved oxygen concentrations in the downgradient well increased substantially and BTEX compounds were completely removed. However, as treatment continued, dissolved oxygen concentrations declined over time, even though hydrogen peroxide was added continually to the system. Oxygen was again detected in this observation well, but only after the amount of hydrogen peroxide added to the treatment stream was increased. This behavior suggested that hydrogen peroxide consumption in the treatment system increased over time, and that this increased consumption led to decreased oxygen delivery to contaminated aquifer sediments.

The microbial processes affecting hydrogen peroxide decomposition in jet fuel-contaminated aquifer sediments were studied in detail by Spain et al. (1989). The delivery system in this study consisted of an infiltration gallery overlying the zone of contamination. Hydrogen peroxide was added to treated ground water, dispersed to the infiltration gallery, and allowed to circulate through fuel-contaminated sediments. Spain et al. (1989) measured rates of hydrogen peroxide decomposition in sediments underlying the infiltration gallery and in recirculated ground water. It was found that sediments rapidly decomposed hydrogen peroxide, with a first-order decomposition rate constant of 7.0 hr^{-1} . This decomposition rate indicated that the half-life of hydrogen peroxide in sediments underlying the infiltration gallery was only about six minutes. This high rate of hydrogen peroxide decomposition, much of which was shown to be catalyzed by microbial activity, greatly decreased the effectiveness of hydrogen peroxide delivery to sediments deep in the zone of jet fuel contamination. This study (Spain et al. 1989) showed that using hydrogen peroxide as an oxidant to facilitate petroleum hydrocarbon bioremediation could be problematic. This, in turn, led some investigators to consider alternative methods for adding oxygen in engineered bioremediation systems.

Another method for adding oxygen to ground water is by means of oxygen release compounds (Borden et al. 1997a). While many chemicals will release free oxygen upon reaction with water, the compounds used in conjunction with bioremediation are mixtures of MgO_2 , CaO_2 , and urea hydrogen peroxide (urea- H_2O_2). Proprietary formulations of these compounds are commercially available (ORC[®], Regenesys Inc., San Juan Capistrano, California) and have been evaluated in the literature. The results of one such test showed that BTEX biodegradation could be enhanced by placing ORC in wells to deliver oxygen to ground water, but that well clogging due to iron oxyhydroxide precipitation also occurred (Borden et al. 1997a).

Nitrate can serve as an energetically favorable electron acceptor under anoxic conditions (Tiedje 1988). Furthermore, by the late 1980s, a number of investigators had shown that petroleum hydrocarbons were biodegraded under nitrate-reducing conditions (Kuhn et al. 1988; Hutchins et al. 1991a). It was logical, therefore, to test the feasibility of using nitrate as an electron acceptor in engineered bioremediation systems. Several early field studies indicated at least partial success in removing BTEX compounds (Batterman 1986; Sheehan et al. 1988; Lee et al. 1988) with nitrate as the primary electron acceptor. These early studies also showed, however, that monitoring the effectiveness of nitrate-based bioremediation was extremely difficult. In particular, it was difficult to separate the effects of hydrodynamic processes (such as dilution) from biological degradation processes on concentrations of contaminants and nitrate. As such, the effectiveness of nitrate-based engineered bioremediation systems remained uncertain.

Faced with these uncertainties, the U.S. Environmental Protection Agency (EPA) initiated a detailed study of nitrate-based bioremediation of a jet fuel-contaminated aquifer in Traverse City, Michigan (Hutchins et al. 1991b). This study was designed to separate the hydraulic effects of circulating ground water from biological effects of nitrate addition. The bioremediation system was built to circulate relatively large volumes of ground water (~200 gallons [~758 L] per minute) through an infiltration gallery. This water percolated to the water table, was transported laterally through fuel-contaminated sediments, and was recovered by pumping wells. This recovered water was then continuously circulated to the zone of contamination through the infiltration galleries. The bioremediation system was also designed to continuously add nitrate to the circulating water. When the system was started up, ground water was circulated for 42 days before nitrate addition was initiated. During this time, a significant amount of aerobic biodegradation occurred due to oxygen added during ground water recirculation. In addition, significant leaching of soluble BTEX compounds was documented. In particular, benzene was almost completely leached from ground water at the site and initially accumulated in the circulating water. There was a net mass loss of 2.9 kg of benzene during the pre-nitrate treatment phase. Much of this mass loss could be attributed to the aerobic decomposition of benzene.

After 42 days of water circulation, nitrate addition to the system was initiated. Concentrations of nitrate initially increased to about 8.0 mg/L and fluctuated thereafter. Concentrations of toluene decreased rapidly in ground water after nitrate addition. Concentrations of *m*- and *p*-xylene also decreased, but at a slower rate. Concomitant with the biodegradation of BTEX compounds, it was shown that numbers of denitrifying bacteria in aquifer sediments increased from about 10^6 to 10^7 cells per gram of dry sediment. In addition, the amount of jet fuel was shown to decrease in aquifer sediments. In shallower sediments (which had been most impacted by recirculating water), concentrations of jet fuel decreased from about 5000 to about 1000 mg/kg. In deeper sediments, however, decreases in jet fuel concentrations were not as substantial. In spite of the residual petroleum hydrocarbons that remained, aqueous concentrations of BTEX compounds were reduced below maximum concentration levels specified by the state of Michigan (Hutchins et al. 1991b).

Engineered bioremediation systems using nitrate as an oxidant have been widely used in other ground water systems (Barbaro et al. 1992; Hutchins et al. 1995; Sweed et al. 1996; Vroblesky et al.

1997). Because nitrate itself in ground water is of regulatory concern, the amount of nitrate added to a contaminant aquifer in some instances was restricted to the 15 mg/L maximum concentration limit for drinking water (Vroblesky et al. 1997). These studies indicate that, in many cases, hydrologic constraints were the principal limitations on the use of the technology. For example, Hutchins et al. (1995) found that nitrate-amended recharge water tended to remediate high-permeability sediments where ground water flow was concentrated. In contrast, nitrate-amended recharge water was not delivered as efficiently to lower permeability sediments and, as a consequence, bioremediation was not as efficient. Vroblesky et al. (1997) reported that hydrogeologic factors, such as seasonal fluctuations in ground water levels and changes in ground water flow directions, affected the efficiency of nitrate delivery to zones of contamination. In addition, Vroblesky et al. (1995) showed that variations in hydraulic conductivity could lead to "stratigraphic trapping" of fuel in the subsurface. Such trapping made it much more difficult to introduce nitrate-amended water to the most contaminated sediments, and was a significant constraint on engineered bioremediation in this system. In other systems, it was found that colloidal material mobilized by recirculating ground water and nutrients could plug aquifer materials, cause significant decreases in sediment hydraulic conductivity, and interfere with bioremediation (Wiesner et al. 1996).

As it became generally recognized that hydrologic constraints had a large affect on the efficiency of bioremediation systems (Baker and Herson 1990; Mercer and Cohen 1990), techniques for maximizing the efficiency of treatment water delivery to the subsurface were investigated. For example, incomplete mixing of injected treatment water with contaminated water in the formation often was found to be a problem (Hutchins et al. 1995). To deal with this, Devlin and Barker (1994) proposed injecting nutrients into a constructed high-permeability wall, and allowing the natural hydrologic gradient to carry the nutrients to the zone of treatment. By *pulsing nutrient injection*, Devlin and Barker (1994) reported that it was possible to maximize mixing efficiency, and thus to maximize delivery of nutrients to the contaminated aquifer.

Microbiologic constraints associated with nitrate-based bioremediation systems were also reported. For example, Barbaro et al. (1992) found that toluene, ethylbenzene, and xylene were efficiently remediated by nitrate addition. Benzene, however, was recalcitrant. Laboratory studies have consistently indicated that benzene does not degrade efficiently under nitrate-reducing conditions (Hutchins et al. 1991a). Because benzene often drives regulatory concern about BTEX-contaminated ground water, this may limit the effectiveness of nitrate-based bioremediation.

Much of the cost associated with engineered bioremediation system designs that are derived from the original Suntech Oil method are associated with drilling and maintaining nutrient injection wells, or constructing infiltration galleries. For this reason, several investigators have considered land surface application systems for remediating contaminated ground water (O'Leary et al. 1995; Sweed et al. 1996). This concept involves pumping BTEX-contaminated ground water from an aquifer, applying this water to surface soils, and allowing it to percolate back to the underlying ground water system. During transport through the soils and unsaturated zone, BTEX compounds are degraded by aerobic microbial processes. In addition, BTEX compounds are volatilized and lost to the atmosphere. O'Leary et al. (1995) reported BTEX mass losses of between 65 and 100% by the time the applied water

reached the water table. Sweed et al. (1996) showed by means of tracer tests that this method might also be used to deliver nitrate to an underlying ground water system. However, a study of nitrate addition to this system indicated that considerable amounts of nitrate were taken up by vegetation at land surface (Hutchins et al. 1995), and that such uptake might reduce the effectiveness of nitrate delivery.

The original Suntech Oil design injected air into the water column of a well to oxygenate ground water. In addition to oxygenating the water, this had the desirable effect of purging volatile and semivolatile hydrocarbons from the water. The combination of adding oxygen to stimulate microbial hydrocarbon degradation while purging contaminants physically from the ground water has developed into a technology called air sparging (Brown 1994). Modern practice of air sparging, however, differs substantially from the original Suntech Oil design. Rather than injecting air into the water column of a well, air is injected directly into a water-saturated aquifer. This technology has been used extensively in Europe (Hiller and Gudemann 1988) and has found widespread acceptance in the United States (Brown et al. 1995).

When dealing with soils and unsaturated zone sediments, oxygen can be introduced by pulling a vacuum as well as by sparging. Soil vapor extraction, as these vacuum technologies are collectively called, has been widely used to remediate soils and unsaturated zone sediments contaminated by petroleum hydrocarbons (U.S. Environmental Protection Agency 1991). A variation of this technology, called bioslurping, has been developed for use with contaminated ground water systems as well (Keet 1995; Connolly et al. 1995). To apply this technology, a well is screened across the water table in a contaminated zone, and a vacuum intake pipe positioned at the water table surface. When vacuum is applied to the pipe, a mixture of oil, water, and soil gas is removed and brought to the surface for treatment. This physically removes free product hydrocarbons floating on the water table surface, but also draws in oxygen through the unsaturated zone which facilitates aerobic biodegradation (Air Force Center for Environmental Excellence [AFCEE] 1994). Because bioslurping combines free product recovery with enhancement of biodegradation processes near the water table, it has proven to be useful (Baker 1995). One of the most useful aspects of the bioslurper design is that it minimizes the volume of contaminated ground water withdrawn from the aquifer, and thereby minimizes the volume of water requiring treatment at land surface. Also, this technology can be applied to fractured rock systems where other oxidant delivery systems are difficult to engineer.

Bioaugmentation, the process of adding specially adapted microorganisms to a system to enhance rates of biodegradation, has been applied to remediating contaminated surface soils and in various ex situ remediation methods (Forsyth et al. 1995; Baud-Grasset and Vogel 1995). However, it has not proven useful for contaminated ground water systems. This largely reflects the hydrologic difficulties inherent in delivering microorganisms efficiently to contaminated aquifer sediments (Thomas and Ward 1994). In one study of a relatively homogeneous sand aquifer, Burlage et al. (1995) reported that the transport and recovery of specific strains of injected bacteria — as identified using PCR amplification of DNA — was much more erratic than that of a dissolved chemical tracer. These researchers suggested that this observed behavior was due to complex patterns of bacterial attachment and disattachment from sediment grain surfaces. It is also possible that the injected microorganisms could not compete successfully with microorganisms

indigenous to the ground water system for available carbon and nutrient resources. Because of these difficulties, and because hydrocarbon-degrading bacteria are ubiquitous in near-surface environments (Atlas 1977), the costs associated with delivering laboratory-grown hydrocarbon-degrading bacteria to contaminated zones are often not justified.

In summary, engineered bioremediation systems for petroleum hydrocarbon-contaminated aquifers are most commonly designed to increase the availability of electron acceptors, usually oxygen or nitrate. In some cases, nutrients such as nitrogen, phosphate, and potassium are added as well. The success of such systems for removing petroleum hydrocarbons is directly related to the efficiency with which the electron acceptor and nutrients are delivered to contaminated water and aquifer solids.

Intrinsic Bioremediation of Petroleum Hydrocarbons in Ground Water Systems

During the 1980s, there was an enormous effort to assess and monitor petroleum hydrocarbon contamination of ground water in the United States and Europe. From this mass of information came several unanticipated and surprising results. It was widely observed, for example, that plumes of petroleum-hydrocarbon contaminated ground water stopped expanding over time and assumed a dynamic steady-state configuration. Perhaps the best-documented example of this behavior was a crude oil spill in northern Minnesota near the town of Bemidji (Baedecker et al. 1988; Cozzarelli et al. 1989; Baedecker et al. 1993). In 1979, an oil pipeline ruptured and spilled 1670 m³ of crude oil onto the land surface. Over the next year, oil migrated downward and formed a lens floating on the water table. The site was instrumented with observation wells and monitored throughout the 1980s and 1990s. A plume of dissolved hydrocarbons, principally BTEX compounds, was observed to develop downgradient of the oil lens. However, by 1985, the BTEX plume had stopped spreading, extending only about 150 m downgradient of the oil lens. Subsequent studies showed that a dynamic steady state had developed between the rate that soluble hydrocarbons were leaching into the ground water, and the rate that anaerobic and aerobic biodegradation processes were consuming the hydrocarbons (Lovley et al. 1989; Baedecker et al. 1993). Thus, after about 1985, the position and size of the BTEX plume did not change significantly. The hydrologic and microbiologic processes causing this observed behavior were subsequently quantified using numerical modeling procedures (Essaid et al. 1995).

Similar observations were being made in other hydrologic systems as well. In a controlled experimental release to a shallow sandy aquifer, Barker et al. (1987) showed that naturally occurring biodegradation processes effectively removed benzene, toluene, and all three xylene isomers from solution in about one year. Barker et al. (1987) used the term "natural attenuation" to describe the combined dilution, dispersion, sorption, and biodegradation processes that caused contaminant concentrations to decrease. Similarly, Chiang et al. (1989) showed significant losses of benzene mass over a three-year period in a contaminated aquifer underlying a gas plant, and attributed this to natural attenuation processes.

On a much larger scale, a study of 7167 municipal supply wells in California showed that, while leaking gasoline was by far the most common contaminant being released into ground water systems, benzene was found in only 10 wells (Hadley and Armstrong 1991). This study concluded that biodegradation processes were actively

consuming BTEX compounds, thereby protecting California ground water supplies from widespread petroleum hydrocarbon contamination.

These and other studies showed that, in many instances, natural biodegradation processes were far more important in preventing the migration of soluble hydrocarbons than had been foreseen in the early 1980s. Because of this realization, a committee of the National Research Council (NRC) first made the distinction between engineered bioremediation, where human intervention was used to enhance microbial processes in ground water systems, and intrinsic bioremediation, where natural microbial processes alone could renovate contaminated ground water (National Research Council 1993).

In their definition of intrinsic bioremediation, the NRC committee also made a distinction between natural attenuation, which occurs ubiquitously, and intrinsic bioremediation, where these natural processes protect human health and the environment. The NRC committee emphasized that intrinsic bioremediation was not a no-action cleanup alternative, but required a rigorous site evaluation to demonstrate that natural attenuation processes could prevent contaminant migration to sensitive receptors such as wells or nearby wetlands. Furthermore, intrinsic bioremediation always requires environmental monitoring to verify that sensitive receptors are not impacted in the future. As such, intrinsic bioremediation could be considered just like any other technology for treating BTEX-contaminated ground water.

Before intrinsic bioremediation could be applied routinely to contaminated sites, however, it was first necessary to develop methods for assessing the efficiency of natural biodegradation processes. Salanitro (1993) emphasized the importance of hydrologic and microbiologic site characterization in assessing natural attenuation processes in aquifers. McAllister and Chiang (1994) pointed out the importance of assessing the availability of potential electron acceptors, especially dissolved oxygen, in contaminated aquifers.

The most extensive effort to develop methods for assessing intrinsic bioremediation was undertaken by J.T. Wilson of the U.S. EPA and the U.S. Air Force Center for Environmental Excellence (AFCEE). The approach developed by Wilson and his associates relied on three independent lines of evidence to establish the efficiency of intrinsic bioremediation (Wiedemeier et al. 1994). These are (1) documenting mass loss of contaminants at the field scale, (2) collecting geochemical evidence of biodegradation processes, and (3) conducting laboratory microcosm studies using aquifer materials collected from the site. This approach was codified into a protocol for use by Air Force personnel at field sites (Wiedemeier et al. 1995a) which has been widely used in the United States.

Many early approaches to assessing intrinsic bioremediation relied on qualitative indicators of biodegradation processes. In order to make such assessments more quantitative, Wiedemeier et al. (1995b) proposed making mass balance calculations based on the stoichiometry of both aerobic and anaerobic biodegradation processes. In this approach, the available supply of oxidants (oxygen, nitrate, Fe(III), and sulfate) were used to calculate the degradation "capacity" of a given aquifer system. Chappelle (1994) proposed that assessments of intrinsic bioremediation could be made quantitative by comparing rates of contaminant transport to rates of biodegradation. To make such comparisons quantitative required consideration of hydrologic (rates of ground water flow and aquifer dispersion characteristics), geochemical (extent of contaminant

sorption), and microbiologic (rates of contaminant biodegradation) factors. If rates of contaminant transport were large relative to biodegradation rates, then contaminant plumes could migrate considerable distances and possibly impact receptors. On the other hand, if rates of contaminant transport were small relative to biodegradation rates, the plume would be more contained. These hydrologic, geochemical, and microbiological factors can be integrated using digital solute transport models (Rifai et al. 1988; MacQuarrie et al. 1990; Chen et al. 1992; Essaid et al. 1995; Chapelle et al. 1995; Landmeyer et al. 1996).

Digital solute transport modeling methods for assessing intrinsic bioremediation rely heavily on methods for estimating petroleum hydrocarbon degradation rates in aquifer systems. Three approaches for measuring biodegradation rates in ground water systems are presently available. The first is to document contaminant loss along a flowpath, subtract out the effects of dilution and sorption, and apply a rate law (usually first order) to the data (Wilson et al. 1993, 1994b; Wiedemeier et al. 1996). Such estimates can also be made by matching field data to time-invariant solutions of the solute transport equation (Buscheck and Alcantar 1995). The second method is to use controlled field tracer studies to estimate biodegradation rates (Stauffer et al. 1994; Thierrin et al. 1995). A third method is to obtain aquifer materials and perform laboratory microcosm studies (B.H. Wilson et al. 1990) or *in situ* columns (Hunt et al. 1995) to estimate biodegradation rates.

Several investigators suggested using both field and laboratory approaches in order to obtain a range of degradation rate estimates (B.H. Wilson et al. 1994). Chapelle et al. (1996) used this dual approach to show that laboratory-measured biodegradation rate estimates are highly sensitive to ambient terminal electron-accepting conditions, and that laboratory conditions must be carefully matched to field conditions to obtain reliable results. In addition, the limitations of assuming first-order kinetics to petroleum hydrocarbon degradation were pointed out by Bekins et al. (1998).

Beginning in the 1970s, methyl tert-butyl ether (MTBE) was added to gasoline. Initially, MTBE was added as an octane-enhancing replacement for tetraethyl lead, which was being phased out of use. Later, MTBE was used as a fuel oxygenate to decrease the amount of carbon monoxide in automobile emissions. The 1990 Clean Air Act Amendments mandated the seasonal use of fuel oxygenates, such as MTBE, in certain parts of the United States. This use has resulted in MTBE being released to ground water (Squillace et al. 1995). Initial research on MTBE indicated that it was relatively recalcitrant to biodegradation. Fujiwara et al. (1984) reported no degradation of MTBE by activated sludge. Jensen and Arvin (1990) reported no biodegradation of MTBE at a concentration of 10 mg/L after 60 days in aquifer sediments, topsoil, or activated sludge. Later, evidence began to emerge showing that MTBE was biodegradable under certain conditions or when certain microorganisms were present. Salanitro et al. (1994) reported an aerobic mixed culture isolated from a chemical plant biotreater sludge that metabolized MTBE to carbon dioxide (40%) and cell biomass (40%). Anaerobic degradation of MTBE under methanogenic and sulfate-reducing conditions was reported by Mormile et al. (1994). Mo et al. (1997) reported three bacterium that could grow slowly in aerobic pure culture using MTBE as a sole carbon source. However, this study also indicated that if alternative carbon sources were available, use of MTBE decreased. Field studies of MTBE biodegradation relative to BTEX compounds (Borden et al. 1997b; Landmeyer et al. 1998) indicate that MTBE does biodegrade in shal-

low aquifers, but that degradation is less than for BTEX compounds. Under Fe(III)-reducing conditions found at many gasoline spills, Landmeyer et al. (1998) reported low but measurable rates of MTBE mineralization. The current consensus of research is that MTBE is biodegradable, but that rates of degradation are low relative to BTEX compounds. This, in turn, has shown that intrinsic bioremediation is less efficient for MTBE than it is for BTEX compounds (Borden et al. 1997b; Landmeyer et al. 1998).

As quantitative studies showed that intrinsic bioremediation could be an effective treatment strategy for BTEX-contaminated ground water, this method became increasingly acceptable to the regulatory community. Prior to 1994, few states considered intrinsic bioremediation to be an acceptable strategy for renovating petroleum hydrocarbon-contaminated ground water. By 1996, however, most states had developed either formal or informal policies for considering intrinsic bioremediation (Ritz 1996). In California, a study by Lawrence Livermore National Laboratory (Rice et al. 1995a, 1995b) recommended that "passive" (i.e., intrinsic) bioremediation be used whenever possible in dealing with leaking underground fuel tanks.

Because of increasing regulatory acceptance, intrinsic bioremediation of petroleum hydrocarbon-contaminated ground water systems has become routine. In some cases, intrinsic bioremediation has been used as a stand-alone method (Rifai et al. 1995; Wiedemeier et al. 1995a; Wilson et al. 1995; Chapelle et al. 1995; Landmeyer et al. 1996). In other cases, intrinsic bioremediation has been used in conjunction with other remediation technologies (Brown et al. 1995). The conjunctive use of intrinsic bioremediation with other more intrusive remediation technologies is likely to see increased application in the future (U.S. Environmental Protection Agency 1997).

The Lessons of History and Hydrogeology

Bioremediation has been used to clean up petroleum hydrocarbon-contaminated ground water systems since the early 1970s. During the next two decades this technology evolved in some ways that were clearly anticipated by early investigators, but also in ways that were wholly unanticipated. The early expectation that addition of electron acceptors would enhance biodegradation of petroleum hydrocarbons (Raymond 1974; Jamison et al. 1975), an expectation that evolved directly from experience with marine oil spills (Atlas 1977), has largely been borne out by subsequent experience (Hutchins et al. 1991b; Wilson et al. 1994a; Vroblesky et al. 1997). Largely unanticipated, however, were the technical problems associated with effectively delivering nutrients and electron acceptors to contaminated ground water and aquifer materials (Kennedy and Hutchins 1992; Vroblesky et al. 1995). Most of these technical problems are directly related to the hydrogeologic complexities and the heterogeneity of ground water systems. These complexities affect the engineered bioremediation of dissolved BTEX plumes, and free-phase sorbed-phase petroleum hydrocarbons (National Research Council 1997). Many widely used technologies such as vacuum extraction (AFCEE 1994), air sparging (Brown 1994), and bioslurping (Keet 1995) were developed in direct response to the difficulties inherent in efficiently adding nutrients and electron acceptors to ground water, and in dealing with free product hydrocarbons.

Several expectations of early investigators have not been borne out by subsequent experience. Bioaugmentation, the process

by which specifically adapted microorganisms are added to stimulate biodegradation, has not attained wide acceptance. This is in sharp contrast to experience with contaminated surface soils where bioaugmentation has proved useful and is an accepted technology (Forsyth et al. 1995). Again, the chief reasons for this are hydrogeologic, and reflect the difficulty and expense of delivering microorganisms to subsurface environments. Many early investigators also anticipated that genetically engineered microorganisms (GEMs) would be an important technology in remediating petroleum hydrocarbons (Chakrabarty et al. 1973). This has not proven to be the case, largely because of public concerns about releasing GEMs into the environment.

Perhaps the most unanticipated technology has been the development, widespread use, and regulatory acceptance of intrinsic bioremediation. Intrinsic bioremediation, the use of natural attenuation processes combined with environmental monitoring as a remedial strategy (National Research Council 1993), is most effective when applied to plumes of dissolved BTEX compounds. In hydrologic circumstances when rates of biodegradation are fast relative to rates of BTEX transport by ground water flow, natural attenuation processes can be highly effective in preventing contaminants from reaching environmentally sensitive receptors (Chapelle 1994). Experience has shown that intrinsic bioremediation of petroleum hydrocarbons is most effective when combined with source removal of free-phase hydrocarbons (U.S. Environmental Protection Agency 1997). Because rates of MTBE biodegradation are lower than rates of BTEX biodegradation in ground water systems, intrinsic bioremediation is less effective for containing plumes of MTBE-contaminated ground water (Landmeyer et al. 1998). A variety of protocols and procedures were proposed to assess the efficiency of intrinsic bioremediation in petroleum hydrocarbon-contaminated aquifers (McAllister and Chiang 1994; ASTM 1995; Wiedemeier et al. 1995a; Chapelle et al. 1995).

The most important lesson that can be gleaned from the history of petroleum hydrocarbon bioremediation is the critical importance of accurately assessing the hydrogeology of each site. Experience has shown that successful application of bioremediation to petroleum hydrocarbons depends on understanding and using the hydrologic characteristics of a given site. As bioremediation techniques are applied to contaminants such as chlorinated solvents, pesticides, explosives, and metals in ground water systems, these hard-won hydrologic lessons need not be repeated.

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