THE EVALUATION OF PUMP-AND-TREAT GROUNDWATER REMEDIATION TECHNIQUES FOR GASOLINE WITH EMPHASIS ON IN SITU BIOREMEDIATION

BY

SHAWN MICHAEL NORTON

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APPROVED:

Thomas Grizzard, Advisor

Harold Post
Harold Post

Adil. N. Godrej
Adil Godrej

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Advisor: Thomas Grizzard
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(ABSTRACT)

The purpose of this research was to evaluate the use of conventional groundwater pump-and-treat technology in the remediation of a gasoline contaminated groundwater, and to discuss the benefits of incorporating biotransformation mechanisms in the groundwater cleanup process. Physical and chemical fate and transport mechanisms occurring in the subsurface were investigated to identify potentially rate limiting phenomena. The overall objective was to describe the processes involved in the conceptual design of an *in situ* groundwater biotreatment system which utilizes naturally occurring microorganisms.

Transport of gasoline in the subsurface as a result of bulk movement and advection was investigated to determine the hydrologic parameters of importance in a groundwater remediation scheme. Saturated and unsaturated flow conditions were included due to the suspected importance of vadose zone storage capabilities. Additional fate and transport phenomena including contaminant dispersion,

adsorption, and vapor transport are discussed as they relate to the cleanup process. Biotransformation processes responsible for degrading gasoline organics are given, along with the rate limiting parameters of a groundwater bioremediation system.

Design of a groundwater remediation system using naturally occurring microorganisms must be preceded by a thorough investigation of subsurface hydrologic conditions, biological conditions, and geochemical properties analysis. The hydrologic properties of interest in the aquifer are obtained through aquifer pumping tests and include aquifer transmissivity, conductivity and storage coefficients. This allows a calculation of well pumping rates and the zone of influence of recovery/injection areas. It further allows a mechanism for determining the suitability of bioremediation utilizing this approach.

Subsurface biological conditions and geochemical properties are closely tied together and are critical to the feasibility of biological groundwater remediation. Demonstration of microbiological culture presence and growth potential is performed utilizing standard microbiological mediums as well as a hydrocarbon medium. Groundwater cation and anion analysis is performed along with nutrient optimization studies to identify design formulations.

Acknowledgements

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SECTION ONE - INTRODUCTION

One of the primary sources of groundwater contamination in the United States has been identified as leaking underground storage tanks (LUSTs) which store gasoline. Current methods for remediating gasoline contaminated groundwater rely heavily on pump and treat operations which are followed by either air stripping of the gasoline components or adsorption of the contaminant onto a granular activated carbon matrix. This pump and treat method involves increasing the localized hydraulic gradient of the groundwater, thereby drawing the free floating gasoline and contaminated groundwater into one or a combination of recovery wells.

Methods for establishing cleanup standards for gasoline contaminants can vary significantly, however, an increasing number of regulatory agencies are utilizing the zero-slope or asymptotic curve approach. This approach holds that the responsible party must demonstrate that contaminants have been removed or contaminant levels stabilized at their lowest concentrations as shown by a plot of contaminant concentration versus time.

Recent information concerning this approach has shown that contaminant concentrations may rebound if monitored after a period of quiescence. The physical and chemical reactions which are believed to be responsible for the rebound effect are complex and any study of these phenomena must include basic concepts of contaminant flow in saturated and unsaturated subsurface zones as well as a discussion of the related factors affecting gasoline mobility such as adsorption, vapor transport, biotransformation, and dispersion.

Gasoline contaminants released from leaking underground tanks are distributed to different phases and locations within the subsurface by transport and partitioning processes. Additionally, these contaminants are acted upon by transformation (biodegradation) processes. The gasoline may be present in any one of four forms: 1) as a free floating liquid gasoline phase 2) as a liquid gasoline and water mixture 3) as a vapor phase, and 4) as a solid phase (adsorbed).

The principal means of remediating groundwater contaminated by gasoline is the use of pumping wells followed by carbon adsorption treatment or air stripping. Pumping wells installed below the water table surface remove water from the aquifer, creating cone-like depressions in the water table into which floating gasoline accumulates. Following this, dissolved gasoline enters the well with the groundwater. This contaminated water is generally sent first to an oil-water separator, and then to the selected treatment method.

The design of a groundwater pump and treat system has historically been conducted by first performing an investigation of the subsurface to establish the characteristics of the aquifer and the gasoline plume. Following this, groundwater pumping rates can be calculated which will theoretically contain the migration of the gasoline. While this method of treatment may be adequate to remove the bulk gasoline as well as some of the dissolved constituents of gasoline, it is clear from the multiphased nature of this problem that a more integrated approach is necessary.

Some of the common deficiencies found in the design of groundwater pump and treat systems consist of the lack of consideration for 1) adsorption/desorption phenomena occurring within the saturated or the unsaturated zone relative to groundwater removal rates, 2) simplified well placement, thereby creating stagnation zones where little or no remediation takes place, 3) the "smearing" of gasoline onto previously uncontaminated areas through the improper design of pumping systems, causing deep cones of depression in the water table, and 4) the vapor phase reservoir that exists in the less than saturated porous zones lying above the water table.

In situ bioremediation, the process of using microbial organisms to break down toxic substances, offers several advantages over conventional groundwater treatment methods. The most important of these includes providing an end of the line to the tracking of hazardous chemicals (as opposed to mass transfer methods). Other benefits include elimination of the observed "rebound effect" from desorption and unsaturated storage release phenomena, potential lower cost, and elimination of the safety issues arising from the multiple handling of hazardous substances such as contaminated soils.

The most common form of bioremediation in use today is aerobic biostimulation. Conceptually, this process involves the use of naturally occurring microbes, whereby the site environmental conditions are altered by the addition of oxygen and nutrients, such that microbial metabolism of the contaminant is enhanced. This results in the breakdown of the contaminant into non-toxic forms. There are additionally two other forms of bioremediation known; bioaugmentation and genetically engineered bioremediation.

Bioaugmentation consists of selecting a bacterial strain known to be a contaminant consumer, adapting them to local conditions such as contaminant concentration, and injecting them into the subsurface. Genetically engineered bioremediation, as implied, uses the concept of gene splicing to develop new bacterial strains capable of degrading contaminants. This paper will focus on the use of naturally occurring microorganisms, as this is the predominant form of groundwater bioremediation currently in use.

This report discusses the aspects of fate, transport, and transformation of gasoline constituents in subsurface aquifers which are fundamental to the design of a groundwater remediation system, and discusses recommended modifications to pump and treat operations. The report further elaborates on the reactions believed to be rate limiting in the complete cleanup of a gasoline contaminated groundwater, and discusses the basic hydrologic and biokinetic parameters that are necessary to engineer an effective bioremediation system. The report will focus on aquifers primarily consisting of sands and gravels as commonly found in the Atlantic Coastal Plain physiographic province.

SECTION TWO - LITERATURE REVIEW

I. Introduction

With the recent understanding that the nation's groundwater supply is one of our most valuable resources, and the knowledge that national groundwater quality is deteriorating in large part by leaking underground storage tanks storing gasoline, intensified research has begun into more efficient and environmentally protective methods of remediating this resource. The total number of underground storage tanks in the United States is estimated at three million, and approximations of those which are leaking have ranged from 10 percent, to as much as 30 percent of this population. Only a limited number of technologies to remediate groundwater are available that have demonstrated performance records, and have progressed to full scale operation. In fact, the two most widely used technologies employed in greater than 95 percent of LUST cases studied were air stripping, and filtration through granular activated carbon following groundwater pumping (EPA, 1988).

Prior to 1972, very little information existed on gasoline contaminated public water supplies. Two spill incidents; one occurring in Forest Lawn, California in 1972 (McKee), and the other in Ambler, Pennsylvania in 1971 (Jamison, _et al_.), prompted increased attention to this problem. In particular, the Pennsylvania site served as a benchmark to gasoline biodegradation research, for this was one of the first attempts at stimulating indigenous microorganisms to degrade gasoline in groundwater. As a result of this research, numerous case studies have since demonstrated the value of biostimulation.

II. Fate and Transport Concepts

The fate and transport of gasoline in the subsurface environment is dependent on a number of physical, chemical, and biological processes. The study of the mechanisms responsible for gasoline migration and transformation primarily began as a result of spill cases observed in the 1970's in California, New York, and Pennsylvania. Prior to this, very little information existed, although much was known about the individual processes theorized to be responsible, including hydrodynamic transport, and chemical and biotic transformation processes.

In 1962, Geraghty discussed the movement of contaminants through geologic formations (McKee, et al. 1972), and theories of petroleum migration in water bearing stratums had been proposed by Schwille and VanDam in 1967 (Abdul, 1988). The major physical processes of importance in groundwater transport have been summarized by Freeze and Cherry (1979). Notwithstanding this information, scientific discussion of the fate and transport of gasoline is a recent phenomena. Bouwer and McCarty (1984) proposed a method of modeling biotransformation in the subsurface which utilizes biofilm kinetics. This has been followed up by Rittman (1980), and others, who also described the concept of a minimum substrate concentration scenario. Dragun (1988), and Keely (1986) have discussed concepts of the subsurface fate of contaminants in a broader context, going beyond petroleum and discussing other hazardous chemicals, and Lyman (1987) has presented extensive data on the fate of gasoline in the subsurface.

Bossert and Botha (1984) presented data on the isolation of hydrocarbon degrading microorganisms. Wilson, et al. (1983) and Ghiorse and Balkwill (1983), reported on results of tests aimed at enumerating and characterizing the bacteria found in aquifers.

Important fate and transport findings are summarized in reports issued by the EPA Office of Water Planning and Standards (1979), the EPA Hazardous Waste Engineering Research Laboratory (1987), and EPA's Robert S. Kerr Environmental Research Laboratory (1987), as well as various reports from American Petroleum Institute (API) and National Water Well Association (NWWA). The U.S. EPA, the API, and the NWWA, among others, have sponsored extensive research in this area. Additionally, numerous case studies have provided the literature with valuable data.

III. Bioremediation Design Concepts

Application of the capacity of subsurface microorganisms to degrade gasoline contaminated groundwater was first demonstrated in the United States by Raymond, et al. (1974). Raymond was awarded a patent on a process designed to remove hydrocarbons from groundwater by stimulating the indigenous microbial population with nutrients and oxygen. Followup studies by Raymond (1978) showed that the supply of dissolved oxygen could limit biostimulation, and alternative sources were sought. In 1986, Raymond received a second patent on a process of utilizing hydrogen peroxide as an oxygen source. Britton (1985) has reported on feasibility studies for the use of hydrogen peroxide to enhance microbial degradation, and Yaniga (1984) has proposed a comprehensive in-situ bioremediation technique which includes elements of hydrologic control, geochemistry, and microbiology.

Raymond's approach, which has since been modified by Yaniga and others, still forms the base of all techniques used for the bioremediation of gasoline contaminated groundwaters. Raymond's design, however, did not provide for treatment of the material above the water table. Additionally, nutrient requirement analyses varied considerably. Based on this, subsequent studies have focused on improving the treatment delivery process, and on maximizing oxygen and nutrient formulations. A system for improved circulation utilizing an injection gallery was proposed in 1985 by Brenoel and Brown (EPA, 1987). Similar versions of this prototype are now being used by most of the environmental firms specializing in this technology, including Dupont Environmental Systems, Groundwater Technology Incorporated, and Westinghouse Environmental Incorporated.

Microcosm studies have been performed by Raymond (1976), Bartha and Bossert (1984), and Litchfield, et al. (1989), to determine optimum nutrient formulations and geochemical limitations. Results have shown that nutrient requirements are site specific, and vary depending on the geological matrix of the spill site.

SECTION THREE - MATERIALS AND METHODS

This report is the result of an investigation into the current methods used in the cleanup of gasoline contaminated groundwaters emphasizing biodegradation. In this section, details of this investigation are given. Primarily, the process utilized in drawing together the multiple scientific disciplines used in the design of an effective groundwater bioremediation system will be discussed.

This research began as a result of intensified interest by environmental regulatory officials into improved methods for remediating gasoline contaminated groundwater. Experience in providing oversight to owners of leaking underground gasoline tanks had revealed that conventional groundwater pump and treat methods were not entirely effective. This was witnessed through the observation of rebounding contaminant concentrations following supposed complete cleanup.

Regulatory officials were contacted in the Mid-Atlantic, Northeast, and Southeast regions of the country to verify the hypothesis that conventional pump and treat technology was not providing adequate treatment levels. A description of the case histories of interest were supplied to these officials. This was followed by independent visits to three state offices to review and retrieve pertinent information. Additionally, a cursorary literature review was performed to identify documented cases of contaminant rebounding.

A comprehensive literature search followed to locate sources of information pertaining to the theoretical aspects of contaminant fate and transport. Information on groundwater pump and treat technology was especially sought. Additionally, information was sought on the conceptual design of in situ bioremediation systems. Federal and private agencies were contacted for this information, including the Environmental Protection Agency, the American Petroleum Institute, and the National Water Well Association.

As more information became available through this search on companies providing bioremediation services, these firms were contacted to provide details of their system designs. Firms contacted included Westinghouse Environmental Services, DuPont Environmental Remediation Services, Groundwater Technology Incorporated, Cambridge Analytical Associates Bioremediation Systems, and IT Corporation

Aquifer Remediation Systems. The final report is a compilation of the multidisciplinary factors which play a role in the design of a bioremediation system.

SECTION FOUR - RESULTS

I. Transport of Gasoline in Subsurface Aquifers by Bulk Transport and Advection

Gasoline migration from Underground Storage Tanks (USTs) in the unsaturated and saturated subsurface environment is controlled primarily by mechanisms of bulk transport, advection, adsorption, dispersion, and vapor transport. The migration that is due to bulk transport and advection can be visualized in three basic stages. These will be discussed first.

Initially, soon after the leak has started, the gasoline distributes itself both vertically and horizontally under the influence of capillary and gravity forces (bulk transport). Typically, this occurs in the vadose zone, which is the geological profile extending from the ground surface down to the upper surface of the water table. The vadose zone is further subdivided into the upper soil zone; the intermediate unsaturated zone and the lower capillary fringe zone.

In stage two of the release, the gasoline has migrated into the capillary fringe zone, forming a gasoline/water/air mixture at the capillary fringe. This zone is characterized by the presence of water at or near saturation. According to Abdul (1988), the gasoline migration in this area is predominantly lateral, and generally in the direction of groundwater flow.

Free floating gasoline is not yet present at this stage. As the leak continues, gasoline continues to accumulate in this capillary zone because the pressure head exerted by the gasoline is not large enough (held under negative pressures) to further displace the water retained in the soil pores in a downward direction. At this stage of the leak, an excessive region of the subsurface can become contaminated and a large volume of gasoline could be stored as residual and capillary held gasoline. A discussion of the mobility of gasoline in this region follows.

When, as in the case of the vadose zone, a porous medium becomes less than fully saturated, the ability of water to pass through the medium sharply decreases. The term given the characteristic ability of an aquifer to transmit a fluid such as water or gasoline is the hydraulic conductivity. Values for hydraulic conductivity range over large orders of magnitude according to the geologic matrix of the aquifer

(Table 1). Values given for conductivity found in the Atlantic Coastal Plain range from 10^{-2} - 10^{2} gallons per day, per foot squared (gpd.ft^{-2}). As the soil begins to desaturate, capillary theory holds that the larger diameter pores will drain first. Since these pores can conduct a large percentage of flow when saturated, their desaturation significantly reduces the fluid transmitting properties of the medium (Abdul, 1988). Many empirical correlations have been developed to confirm this.

Mathematically, this empirical relationship between hydraulic conductivity and percent saturation is given as follows:

(see next page)

$$K_e = K_o \, S_e^{\,n}$$

where: K_e = effective hydraulic conductivity ($gpd.ft^{-2}$)

K_o = saturation hydraulic conductivity ($gpd.ft^{-2}$)

S_e = effective saturation (percent)

n = variable exponential factor ranging between 3-4 in the literature.

Using this, steady state flow rates of water or gasoline in the unsaturated zone can then be determined from a modified form of Darcy's law. Darcy, in his water supply paper of 1856, reported on experiments of the flow of water through sands. He found that the flow of water in a column of sand was proportional to the head loss due to friction, and inversely proportional to the thickness of the sand through which the water passed. In other words, flow varies directly (increases or decreases) with head loss due to friction, and decreases with increasing thickness of the aquifer matrix. This modified form for unsaturated flow considers the relationships between hydraulic conductivity and cross sectional area, as well as the gradients in unsaturated soil due to capillary and gravitational forces

(Figure 1). Mathematically this is given as:

$$Q = K_e A \frac{(Hc - Z)}{Z} \pm \frac{(dh)}{(dl)}$$

where: Q = flow (gal/unit time)

K_e = effective hydraulic conductivity ($gal.day^{-1}.ft^{-2}$)

$\frac{Hc - Z}{Z}$ = gradient due to capillary forces

A = area (m^2)

$\frac{dh}{dl}$ = gradient due to gravity

The plus or minus sign given in the equation is for upward (-) and downward (+) directions. For movement in a vertical direction, the gradient due to gravity is 1/1 or 1. For lateral or horizontal movement in the unsaturated zone, the gravitational term can be eliminated. Typical values of capillary rise in medium sands are approximately 10 inches (250 millimeters), (Abdul, 1988).

The impact that gasoline trapped in the unsaturated zone has on a remediation system will be discussed in detail in subsequent sections. It is important to note, however, that this impact is significant and should be a factor to consider in any remediation effort.

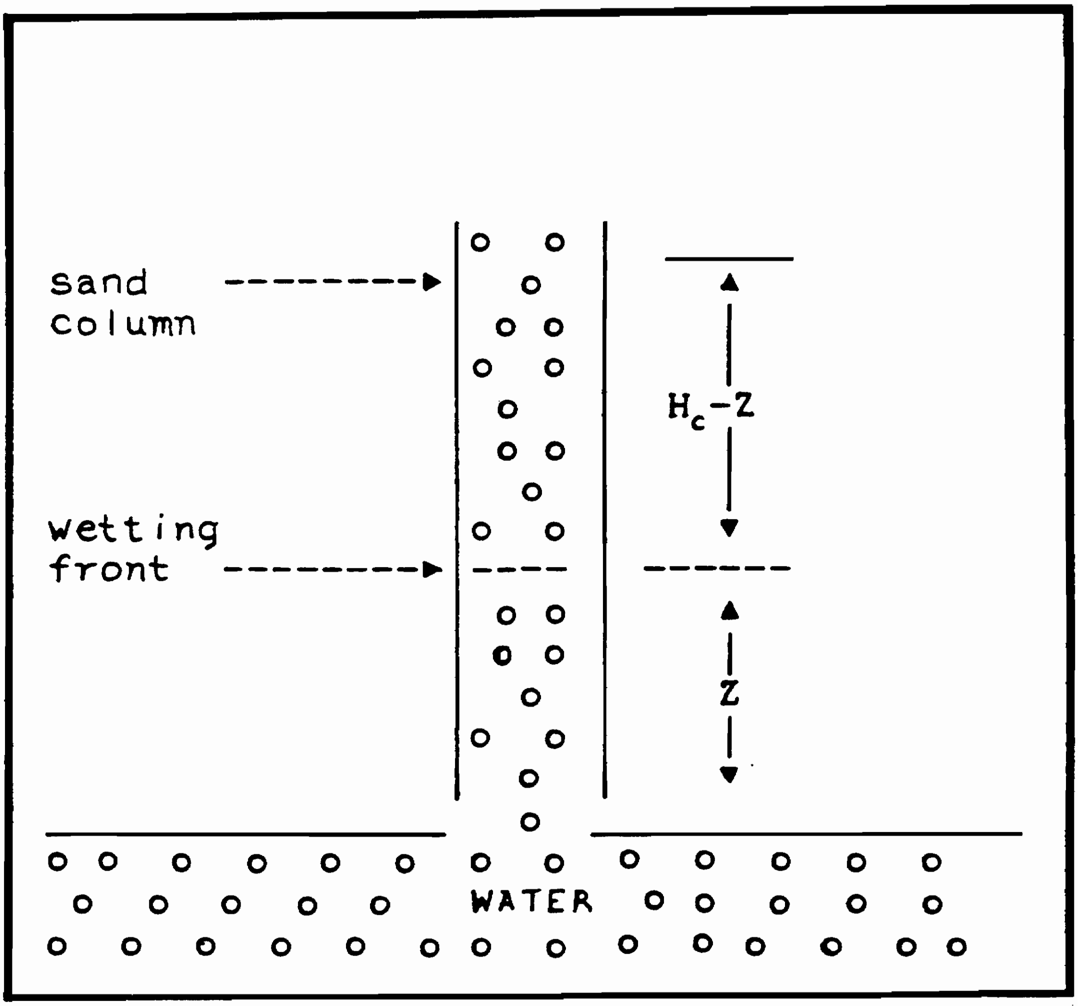
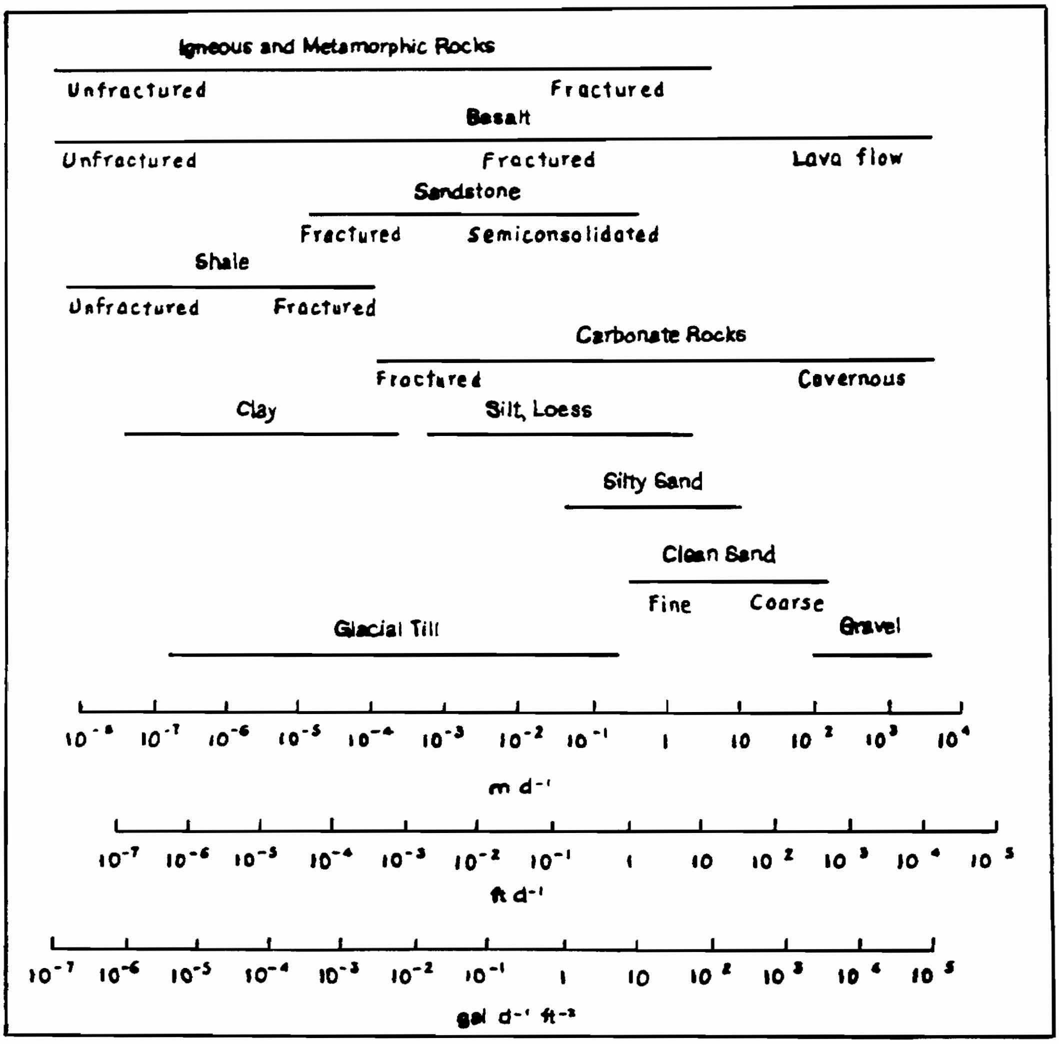


Figure 1 Diagrammatic Representation of Capillary Forces

Table 1. Hydraulic Conductivity of Selected Rocks, EPA (1985)



Stage three of gasoline movement in the subsurface constitutes the accumulation of free floating gasoline on the top of the water table (advection and dispersion). Because this accumulation is no longer under negative pressure of capillary conditions, the gasoline is now free to flow. The migration of the gasoline is again primarily lateral and in the direction of groundwater flow.

The flow of the gasoline which is floating on the water table generally occurs under near ideal Darcian conditions. Under these conditions, flow is non- turbulent, and the head loss due to friction varies directly with groundwater velocity. Mathematically, this is expressed as:

$$Q = KA \left(\frac{dh}{dl}\right) \quad \text{or} \quad Q = KA\,I$$

where: $I = {}^{dh}/_{dl}$ = slope of hydraulic gradient (dimentionless)

K = constant of hydraulic conductivity ($gpd.m^{-2}$)

A = cross sectional area (m^2)

Q = flow (gal./unit time)

The rate of groundwater movement (velocity) in the saturated zone has been derived from a combination of Darcy's law, standard hydraulics velocity equations, and the addition of a term for porosity (O_e). This addition is necessary because the water or gasoline will only move through porous openings in the subsurface. Velocities calculated with this equation are average values. Adding the porosity term, it follows:

if; $Q = \frac{KAI}{O_e}$ and $Q = AV$

then; $AV = \frac{KAI}{O_e}$

and; $V = \frac{KI}{O_e}$

II. Dispersion, Adsorption, and the Vapor Transport of Gasoline in Subsurface Aquifers

A. Introduction

The previous discussion of gasoline movement in the unsaturated and saturated zones describes bulk transport and advection. A discussion of the fate of that portion of the gasoline spill which is transported by other methods is also essential to any remediation effort.

Gasoline that contacts the saturated zone is generally found as free floating product, or as dissolved product in the groundwater as discussed previously. Gasoline that remains in the unsaturated zone is generally partitioned among four phases; as free liquid, as vapor in the soil air, as dissolved product in the soil water, and as gasoline components adsorbed onto soil particles (Figure 2). Gasoline may also be adsorbed to the soil in the saturated zone, however, this is to a lesser extent. Distribution of gasoline components among these phases results from soil-air and water partitioning, dissolution and solubility in groundwater, and sorption between water - soil boundaries. Each of these fate and transport mechanisms are discussed below.

B. Adsorption

The extent to which gasoline compounds adsorb to a soil is primarily dependent on the organic carbon content of the soil particles and on the sorption potential of the chemicals in gasoline. The texture, structure, moisture content, Ph, and cation exchange capacity of the soil also play a role in this transfer.

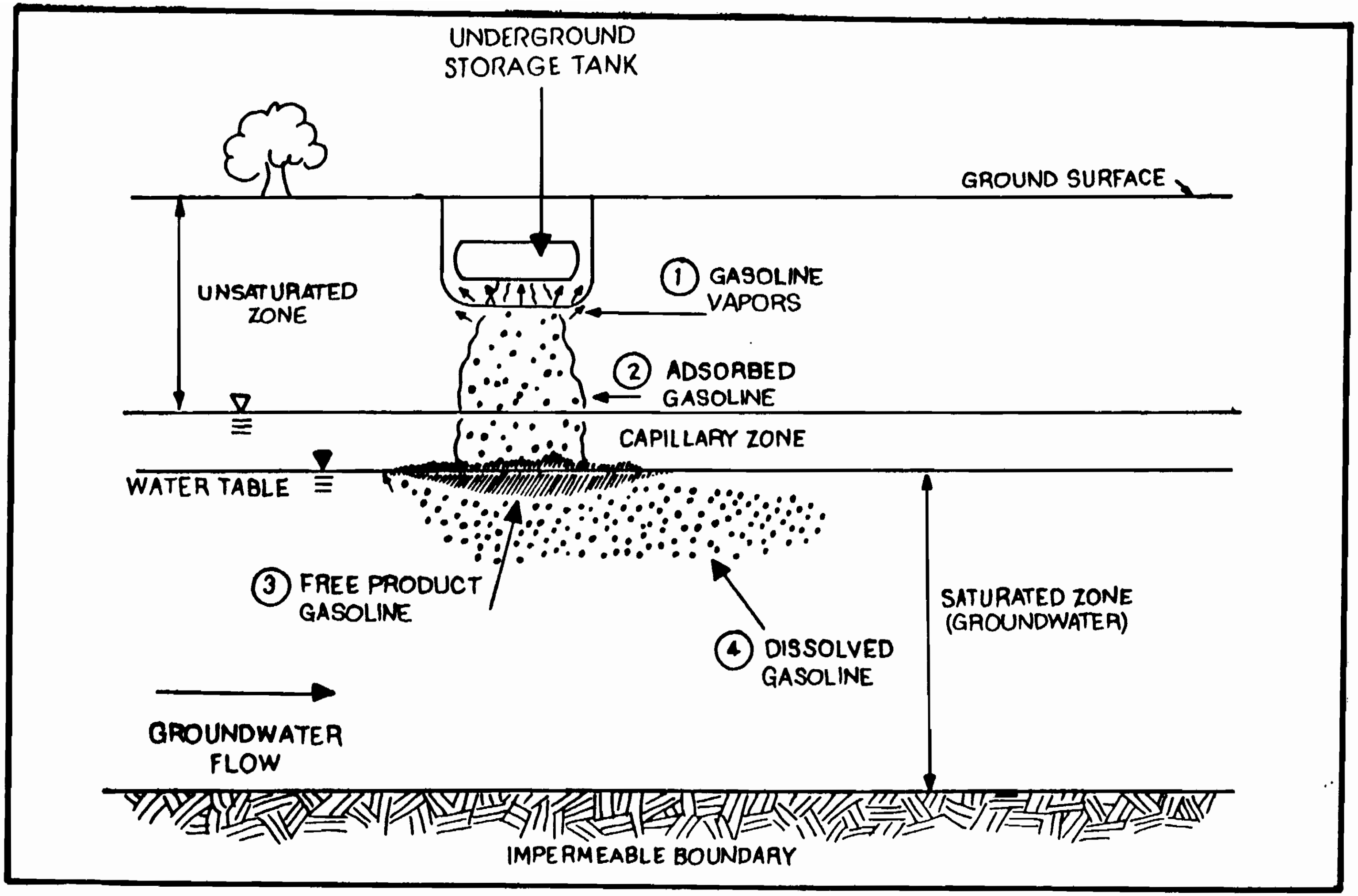


Figure 2 Schematic of Subsurface Environment and Four Phases of Contamination

Adsorption of gasoline constituents in the unsaturated and saturated zones can generally be described by an equation which relates the partitioning of the gasoline between the soil and water phases. This equation is generally written as:

$$Kd = Cs/Ce$$

where: Kd = partitioning coefficient

Cs = concentration in soil

Ce = concentration in water

Use of this equation to describe gasoline adsorption implies that all adsorption sites present on soil surfaces have a fairly constant affinity for the gasoline and that chemical equilibrium conditions exist. According to Dragun (1988), the condition of adsorption site affinity generally exists over a limited range of chemical concentrations which were used to determine the constant, Kd. Lyman (1987) asserts, however, that the assumption of local equilibrium is reasonable because adsorption-desorption rates are rapid and the rate of removal of gasoline from the local environment can be slow relative to site assessment and cleanup time scales.

Figure 3 depicts the adsorptive properties of petroleum in groundwater and the subsequent limitations to pump and treatment effectiveness. Dragun (1988) and Keely (1989) note that when chemical adsorption-desorption processes are slow relative to water movement, the desorption process is generally the one which is incomplete, and a rate constant is usually necessary in the equation to correct for this. Adsorption of gasoline to soil particles is a reversible process that releases the gasoline in an unchanged form (unless biotransformed) after temporarily holding it. In the groundwater remediation field, this effect is commonly termed retardation. Under actual conditions, retardation implies that the gasoline is not moving as rapidly as the groundwater, and in fact being retained on, or between the soil particles.

As stated earlier, a soils organic content is an important parameter in determining the partitioning of gasoline. Many researchers have attempted to correlate values of Kd on the basis of soil organic carbon content. Mathematically, this has been expressed by Dragun (1988) as:

$$Kom = kd/om \quad \text{or} \quad Koc = Kd/oc$$

and: $Koc = 1.724\ (Kom)$

where: Koc = soil adsorption coefficient normalized for soil organic content

oc = soil organic content $\frac{(\text{mg oc})}{(\text{mg soil})}$

om = soil organic matter content

Kom = soil adsorption coefficient normalized for soil organic matter content

Typical values of soil adsorption coefficients and water solubility for gasoline constituents are given in Table 2.

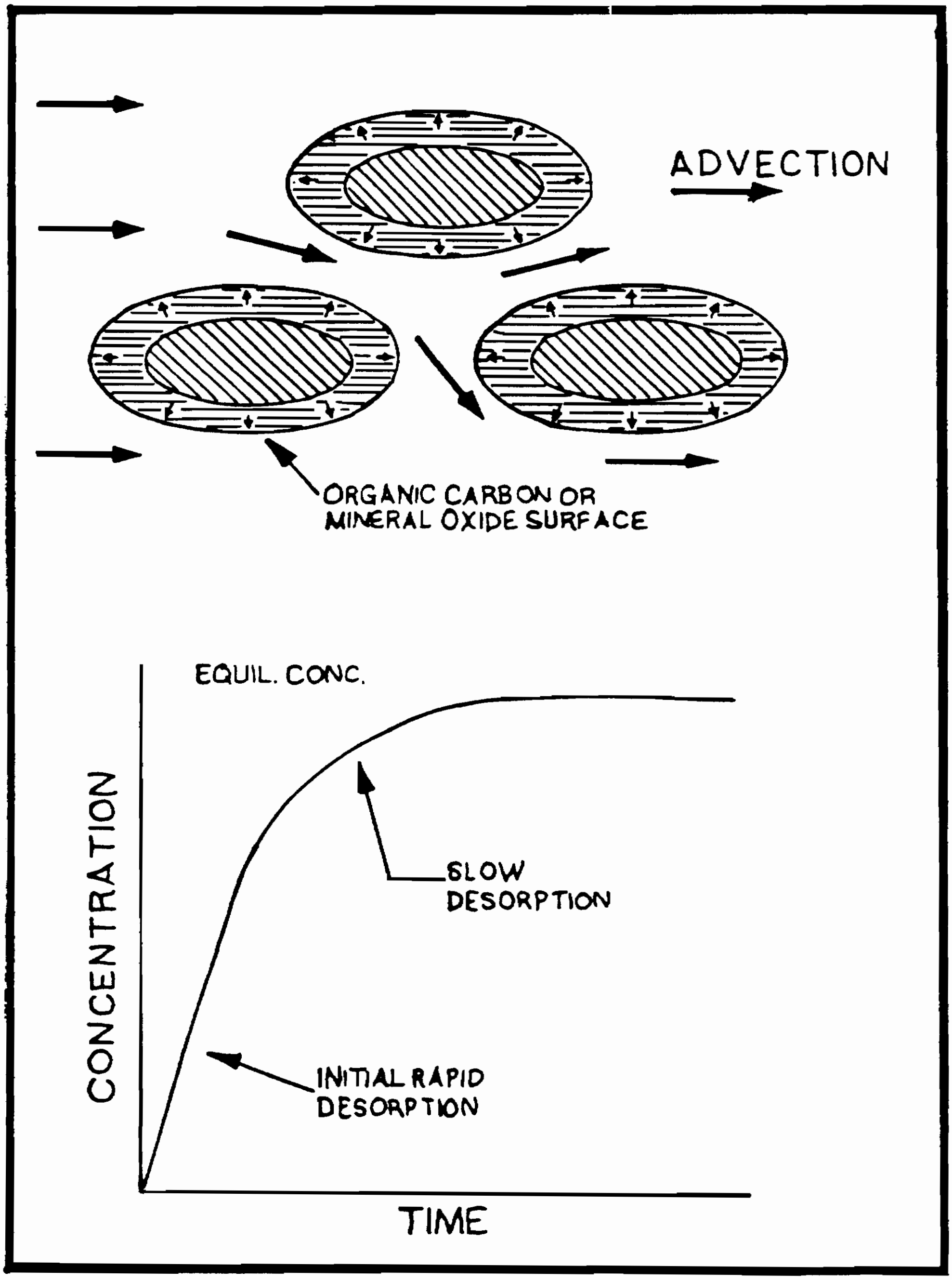


Figure 3 Sorption Partitioning Limitations to Pump and Treat Effectiveness

Table 2. Properties of Selected Gasoline Compounds (Lyman, 1987)

Chemical	Water Solubility	Koc Value
(n) Heptane	2.68	4,300
(n) Hexane	12.5	1,900
(n) Pentane	41.2	910
Ethylbenzene	167	680
Toluene	537	380
1-Pentene	148	460
(m) Xylene	162	720
Benzene	1,780	190
Dodecane	0.005	88,000
1,3,5 Trimethyl - benzene	72.6	940

Note: Solubility given as mg/L.
Koc Values given as ml/g.

C. Dispersion

The movement of gasoline which has dissolved in groundwater can be of a mechanical nature (advection) as previously described by Darcy's Law, or of a chemical nature, that which occurs on a molecular level. This molecular transport is known in groundwater modeling as dispersion, and can be a factor in old gasoline contaminated sites where enough time has allowed for extensive molecular diffusion.

When liquid gasoline and water come in contact, the extent of the dissolution of gasoline constituents will vary depending on their individual solubilities. The hydrocarbon constituents with highest solubilities as shown in Table 2 are the light aromatics (benzene, toluene, xylene, and ethylbenzene). Many gasoline additives, such as ethylene dibromide, ethanol, and methanol, have higher solubilities, but are less toxic, and are therefore of less concern. According to Lyman (1987), the solubility of gasoline in water is not equal to the sum of the solubilities of the individual gasoline constituents, but is much lower (255 mg/liter at 20° centigrade.

Generally speaking, flowrates for groundwater are slow (non-turbulent), and the process of dissolution is also slow, and is usually dependent upon the chemical partition coefficients and on molecular diffusion across gradual concentration gradients. The effects of dispersion explain the observation that contaminants occupy a greater extent of the saturated zone than can be due to advection only. Diffusion coefficients in quiescent systems for neutral organic chemicals in water are all close to 10^{-5} centimeters squared, per second (cm^2/s) at 25°c ; thus, dissolution rates for different constituents should be similar (Lyman, 1987).

D. Vapor Transport

In the underground storage tank environment, gaseous movement can be a significant component of overall migration of leaked product, particularly if that product is highly volatile, as in the case with gasoline. Movement and storage of compounds in the vapor phase through advection or diffusion or both processes can occur in all directions from a leak source.

The gaseous behavior of gasoline is largely a function of the diffusivity and permeability of the vapor in the porous media of concern, and its distribution coefficients which describe interactions with soil particles and moisture. The potential for contamination of soils and ground water in a particular tank location depends on the combination of these parameters at the site. Additionally, the soil and rock formations must be sufficiently dry to permit interconnection of the soil pores.

Gaseous diffusion is a process in which the components of any gas-filled space will eventually become thoroughly mixed. If the density of the diffusing substance is different at different points, diffusion will take place from points of greater to lesser density, and will not cease until the density at all places is the same. Therefore, it follows that gaseous diffusion of each gasoline component in the subsurface will occur whenever there is a difference in its concentration between the soil and outer atmosphere, or at points within the soil because of irregularities in the consumption and release of gases. Diffusion coefficients for hydrocarbons in air at 25°c and 1 atmosphere range from 0.09 centimeters squared per second cm^2/s to 0.06 cm^2/s (Lyman, 1987).

Vapor pressure is a very strong function of temperature. The importance of this effect is significant because this relationship between temperature and vapor pressure is nearly exponential (Lyman, 1987). In soils where temperatures are affected at a depth consistent with contamination, volatilization could be increased.

Vapor advection (also called bulk flow or convective flow), is a transport process in which a gas moves in response to a pressure gradient. Unlike diffusion, advection occurs as bulk flow, the mixture of gases having no tendency to migrate according to the concentrations of its separate gaseous components and therefore, it behaves as one gas. This is because any diffusive effects that would vary the movements of individual gases according to their concentration are minor in comparison to the overall pressure gradient flow. In advection, the gas acts as a fluid continuum driven by the pressure gradient. Movement of gases in the unsaturated zone through advection is governed partly by the vapor permeability of the porous medium and partly by the air pressure created by the gaseous source. Other factors which could cause pressure gradients influencing advection include barometric pressure changes, rise and fall in water table, wind fluctuation, and rainfall percolation.

III. Biotransformation Processes

A. Introduction

The process of transforming the chemical components of gasoline found in groundwater to non-toxic forms and ultimately to carbon dioxide, water, and biomass has been observed to be predominantly a function of microbial metabolism. Heterotrophic bacteria are the most common group of microorganisms providing the metabolism of the gasoline organic, although a few other life forms have been detected. Heterotrophs, by definition, are those organisms which utilize the organic substrate as both a carbon and energy source. In this scenario, a portion of the organic material is oxidized to provide energy, and the remainder is used to build biomass.

The reactions which result in the utilization of organic substrate are controlled by microbially produced enzymes. Microbial enzymes act in three general types of chemical reactions: hydrolytic, oxidative, or synthetic, and can be of the intracellular or extracellular type. Critical among these reactions are the energy producing reactions, which involve oxidations and reductions, and provide energy for the maintenance and growth of microorganisms.

The activity of enzymes, as well as the overall growth processes of the microorganisms, is influenced by the physical environment which surrounds them. The most important of these environmental factors are: oxygen and nutrient availability, temperature, and pH. Additionally, the presence of certain chemical ions can effect the activity of enzymes by accelerating the reaction or inactivating the enzyme.

Depending upon oxygen utilization, or more specifically, the gasoline degraders preferred electron acceptor, microorganisms can be classified as aerobes, anaerobes, or facultative organisms. Where oxygen is used, these are classified as aerobes, whereas organisms that use some other molecule as their electron acceptor are anaerobes, and those that can use both are termed facultative. Fermentation, another form of degradation, does not utilize an electron acceptor, and therefore is classified independently.

Gasoline is made up of a complex of different aliphatic, and aromatic hydrocarbons. Microorganisms found in the subsurface have the ability to discriminate, or preferentially utilize these compounds, and attempts at predicting the fate of these chemicals have revolved around biofilm (Monod) type kinetics. This predictive model has been supplemented by a theory of cometabolism, which helps explain lower concentration levels than would be predicted by Monod kinetics.

B. Microbial Characterization of the Subsurface

Until recently, it was assumed that the subsurface areas below the plant root zone, were for all practical purposes, sterile. This perception was the result of early studies on the stratigraphic distribution of microorganisms in soils by Ehrlich and others, which indicated that microorganisms concentrations dropped off significantly with soil depth (Wilson, et al. 1983). This theory was bolstered by the fact that pristine groundwaters are generally considered to below in organic carbon content, therefore limiting microbial populations. As a result of improved aseptic soil sampling techniques, however, as well as improved analytical techniques such as epifluorescence microscopy, it is now known that this is not the case.

Numerous research initiatives have now confirmed the presence of extensive bacterial populations at significant depths in the subsurface. Jamison, Raymond, and Hudson (1975) had reported that high concentrations of bacteria were found where the concentrations of gasoline was high. In a study conducted by Ghiorse and Balkwill (1983) in Fort Polk, Louisiana, densities of 10^6 organisms per gram of dry soil were found in uncontaminated soils. This study was performed to verify the existence of subsurface microbial populations and to compare Acridine-Orange fluorescent measurement techniques to standard, direct count measurement culture techniques. The results given by Ghiorse and Balkwin using the fluorescent method (10^6/g) are significantly greater than the plate count method, which yielded less than 100 colony forming units (CFU) per gram dry weight.

In a subsequent study by Wilson, et al. (1983), at a site in Lula, Oklahoma, the numbers of fluorescent bacteria were found to range between 3.4 - 9.8 x 10^6 organisms per gram dry soil, at three different depths. This was compared against plate count measurements, which yielded generally much lower and varied results, ranging from 8 x 10^3 organisms/g to 3.4 x 10^6 organisms/g. Novak, et al. (1989) additionally reported on bacterial density measurements at five sites which showed that the fluorescent technique yielded higher results, ranging in magnitude as much as 3 orders above plate counting techniques.

Subsurface aquifers that have not been contaminated by organics are generally considered to be nutrient poor (oligotrophic). This is due to the breakdown of the organic substances originating at the surface as they migrate through the biologically active soil zone. According to Wilson et al. (1983), although both gram- positive and gram-negative forms exist, gram-positive bacterial forms predominate in such nutrient poor environments due to their small size and coccoid shape. This theoretically yields a large surface to volume ratio, and should allow these organisms to take up nutrients from dilute solutions more rapidly. Ghiorse and Balkwill (1983), reported very similar results, finding that less than one-third of all bacterial forms observed in an uncontaminated aquifer were of the gram-negative form, indicating a large gram positive population.

In contrast to this, aquifers which have been contaminated by organics appear to have both gram negative and positive forms, with greater numbers of gram-negative bacteria. In a study conducted by Sun Ventures, Incorporated, on the stimulation of bacterial activity in groundwaters containing petroleum, 19 of 32 total species identified were of the gram negative variety. Additionally, 12 of these were of the Pseudomonas or Acinetobacter genus (API, 1975). In additional work performed by Smith (1985), it was found that a greater number of gram-negative species existed in a creosote contaminated sample, as opposed to a non-contaminated sample from the same site (EPA, 1987). In bacteriological investigations conducted by McKee et al. (1972), Pseudomonas and Arthrobacter were shown to utilize gasoline as a source of energy for rapid growth in the presence of trace nutrient salts and adequate dissolved oxygen. It is interesting to note that at both contaminated and uncontaminated sites, the predominant bacterial forms found were of the species Pseudomonas and Nocardia.

EPA (1987) has reported on studies conducted by Jamison *et al*. (1976) which suggest that *Pseudomonas* was responsible for aromatic degradation, while *Nocardia* cultures were responsible for the degradation of the aliphatics. It is further interesting to note that *Pseudomonas* tends to be a rapidly growing organism, whereas *Nocardia* is not (API, 1975).

Other bacterial species which were isolated during Raymond's and Jamison's work include *Micrococcus* and *Flavobacterium*. Some of the constituents of gasoline that did not support the growth of any specific bacterial species were the branched paraffins, olefins, or cyclic alkanes. One hypothesis for the degradation of these is cometabolism of the organics, which will be discussed in subsequent sections.

C. Biodegradation Pathways

Microbial degradation of gasoline can occur by three general methods; aerobic respiration, anaerobic respiration, or fermentation. In aerobic respiration, the gasoline substrate is broken down by a series of enzyme mediated reactions in which oxygen serves as an external electron acceptor. In anaerobic respiration, the gasoline substrate is also broken down by enzyme mediated reactions, however, other compounds such as nitrates, sulfates, and carbon dioxide, serve as the external electron acceptors. Finally, in fermentation, the gasoline substrate is broken down again by enzyme mediated reactions, but without the use of an electron transport chain, using instead the organic material as the electron donor and the acceptor.

As stated, aerobic respiration is the process whereby oxygen is utilized as the terminal electron acceptor in a series of enzyme mediated chemical reactions, and is reduced to water. The importance of oxygen as the electron acceptor is the completeness of the oxidation reaction, and the subsequent amount of energy that becomes available. According to Benefield and Randall (1980), aerobic microorganisms tend to oxidize their food completely, and yield far more energy in the process. Additionally, aerobic degradation of hydrocarbons is enhanced by the action of oxygenase enzymes and, therefore, the presence of free oxygen.

Heterotrophs recover the energy released during the oxidation of organics by two methods; Substrate Level Phosphorylation (SLP), and Oxidative Phosphorylation (OP).

In the SLP reaction, a portion of the energy released during the oxidation process is used to convert Adenine Diphosphate (ADP) to Adenine Triphosphate (ATP), thus forming high energy phosphate storage bonds. In the OP process, electron pairs produced by the oxidation of an electron donor in the citric acid cycle are passed through an electron transport chain, releasing energy again to convert ADP to ATP. The energy recovered is directed towards biomass growth and cell maintenance.

Gasoline is made up of four major groups of hydrocarbon compounds: alkanes (paraffins), alkenes (olefins), cyclohexanes and cyclopentanes (naphthenes), and aromatics (Lyman, 1987). In addition to this, gasoline can have several types of additives, including alcohols, ethers, and phenols, which are used as either octane enhancers, detergents, or antioxidants, among other things. It is generally accepted that of these compounds, the aliphatics are more readily biodegraded than the aromatics due to the carbon bond structure of these compounds.

According to Atlas (1987) the transformation pathway observed for the degradation of the alkane series proceeds from a straight or branched n-alkane to a primary fatty alcohol. This is further oxidized to a fatty aldehyde, which is followed by a series of fatty acids, sequentially two carbon atoms shorter (C_{12} to C_{10} to C_8). He also notes that alkanes in the C_5 - C_9 range are toxic to many organisms. Bartha (1986), asserts that this is due to the solvents ability to disrupt lipid membranes. The C_1 - C_4 range are limited in their biodegradation potential to only a few organisms. This is in contrast to the C_{10} - C_{22} range, which are readily degraded.

The cyclohexanes, as well as the alkenes, tend to be more toxic and more persistent than the alkanes, thus making them more resistant to biotransformations. This does not preclude them from being broken down. A mechanism which has been proposed for their breakdown is termed cometabolism (Atlas, 1987 and Bartha, 1986). This is a process whereby one hydrocarbon compound provides the carbon and energy a microbe needs to survive, allowing the microbe to feed on a secondary substrate, even though it is not preferential. According to Bartha (1986), cyclohexanes are transformed by a not fully characterized oxidase system to a cooresponding cyclic alcohol, which is dehydrogenated to Ketone. This ketone structure is then opened by a lactone hydrolase enzyme.

The process identified by Atlas, as well as Bartha, for the microbial degradation of the aromatics involves the introduction of oxygen into the ring through a dioxygenase enzyme attack. This converts the aromatic to a dihydrodiol, which is further oxidized to a dihydroxy product. The ring substance is then ortho or meta cleaved yielding ultimately tricarboxylic acid intermediates. Bartha asserts that aromatics with more than four condensed rings do not serve as a substrate for microbial growth, though they may be subject to cometabolic transformation. Hydrocarbons, being fully reduced substrates, require an electron sink to be utilized. In the initial attack on a hydrocarbon, oxygen probably serves as this sink and is theoretically derived from water (Bartha, 1986). However, in subsequent reactions, other external electron acceptors may be used (anaerobic respiration) or as is the case in fermentation, no external electron acceptors are required.

Taking the case of anaerobic respiration, inorganic compounds which can serve as the external electron acceptor include carbon dioxide (CO_2), nitrates (NO_3^-), sulfates (SO_4^{2-}), and manganese and iron (Mn^{2+},Fe^{2+}). When CO_2 is reduced, it forms methane (CH_4); and in the case of NO_3, it can be reduced to various forms, including ammonia (NH_3), nitrous oxide (N_2O), or molecular nitrogen (N_2). When sulfates are reduced, they form hydrogen sulfide (H_2S), whereas the reduced forms of Mn^3+ and Fe^3+ are given as Fe^{2+} and Mn^{2+}. The sequence of microbial processes that occur as environmental conditions change from aerobic to anaerobic usually follows the pattern of: aerobic respiration, denitrification, manganese and iron reduction, sulfate reduction, and methane formation (Lee, et al. 1988).

It is generally accepted that the metabolic pathways followed in the breakdown of a carbon substrate are the same for both anaerobic and aerobic respiration (Benefield and Randall, 1980). The major difference between these processes is the amount of energy produced as a result of oxidative phosphorylation and, thus, the amount of energy which is assimilated into cell growth. In addition, rates of degradation under anaerobic conditions are typically slower than those under aerobic conditions (Lee et al. 1988).

In a study conducted by Dunja and Vogel (1986), greater than 50 percent of available benzene and toluene was reportedly degraded in methanogenic cultures. However, this was only after an initial acclimation period of 90 days. Wilson et al. (1986), also determined that benzene, toluene, and xylenes could be degraded under anaerobic conditions, however, the process was considerably longer (8 weeks) than that observed under aerobic conditions (2 weeks). Britton (1989), reported favorably on the addition of nitrate as an external electron acceptor to oxygen limited systems in the degradation of phenol. Novak et al. (1989), however, suggests that nitrate addition decreases degradation rates except in soils with a high pH, due to the accumulation of nitrite, which is toxic.

In describing the last type of gasoline degradation; fermentation, the process relies on an initial endergonic reaction (energy consuming), whereby energy is obtained from cleaving a terminal phosphate group from ATP molecules, to begin the transformation process. Following this, substrate level phosphorylation does occur, however, as no external electron acceptors are present, the chemical compound "pyruvate" is not converted to acetyl coenzyme-A, and the reduced substrate does not enter the electron transport chain. The net energy yields from this process are significantly lower than aerobic or anaerobic processes. This results in very low biomass production and, therefore, very slow biotransformation rates. According to Bartha (1986), little or no hydrocarbon metabolism occurs in strictly anoxic sediments.

D. The Kinetics of Biodegradation

The subsurface environment is generally characterized by low organic substrate and nutrient concentrations and high specific surface area, which favors the predominance of bacteria attached to solid surfaces in the form of biofilms (EPA, 1987, from Wuhrmann, 1972). According to this theory, the attached bacteria are generally fixed in one place and obtain energy and nutrients from the groundwater that flows by. These bacteria are actually present as microcolonies (Lee, et al. 1988). Groundwater that flows through the contaminated region of the aquifer will continue to supply oxygen, if available, and other electron acceptors to the microorganisms. Because of this, researchers have investigated the use of biofilm kinetics to describe the transformation of organic pollutants in groundwater.

A picture of a biofilm as used here would consist of two layers (Figure 4), attached to a supporting surface. Working from the inside out, the first layer would consist of the biofilm, with a given cell density and cell thickness. The outside layer is then the diffusion or liquid film layer. Within the biofilm, two processes occur simultaneously; utilization of the substrate by the bacteria, and diffusion of the substrate through the biofilm.

Mass transport into the biofilm is governed by Fick's Law of diffusion as follows (Rittman and McCarty, 1984):

(see page 52)

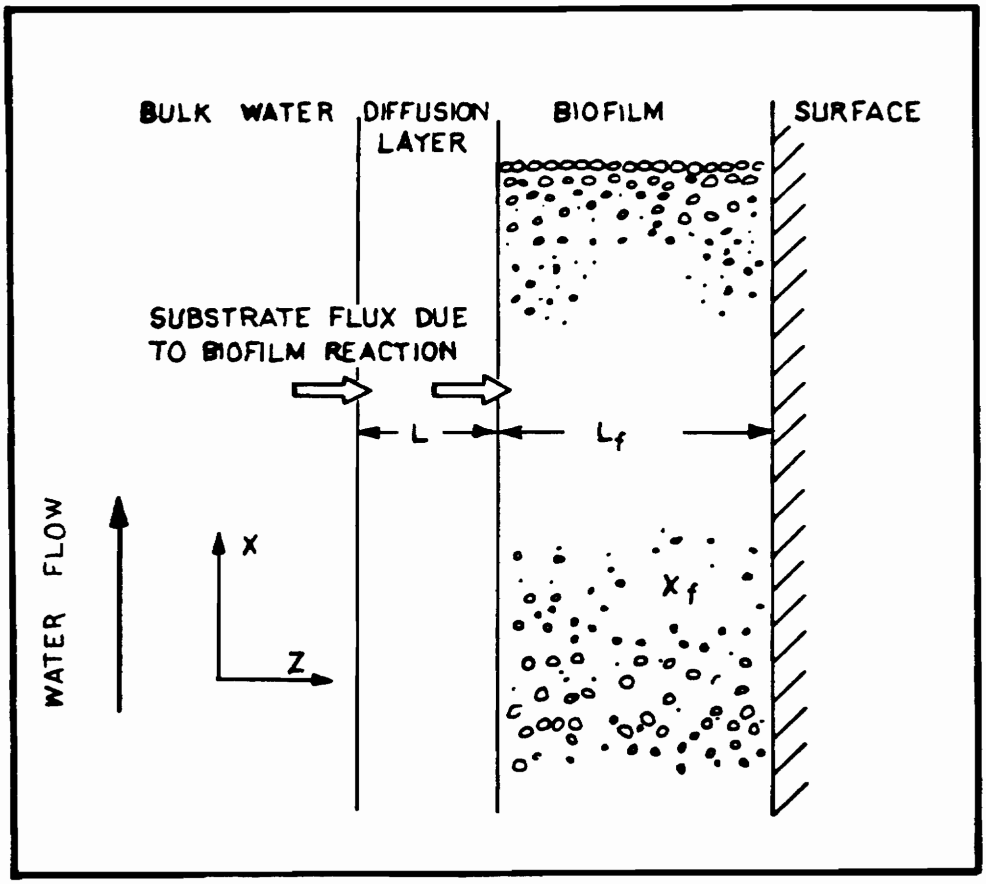


Figure 4 Idealized Biofilm Illustrating Uniform cell Density, Thickness, Water Flow and Substrate Flux into the Biofilm

$$J = -D \cdot \frac{dsf}{dz} = D \cdot \frac{S - S_s}{L}$$

Where: J = flux of rate limiting substrate

D = molecular diffusivity of rate limiting substrate in water

Sf = concentration of rate limiting substrate at a point within the biofilm

S = concentration of rate limiting substrate in bulk fluid

Z = distance normal to the surface of the biofilm

L = diffusion layer thickness

Ss = concentration of rate limiting substrate at biofilm surface

The diffusion of the substrate into the biomass is a rate limiting process affecting biomass growth. Other factors affecting biofilm thickness include: groundwater flowrate, substrate concentration, and the rate of substrate utilization of the microorganisms.

Rittman and McCarty (1980) have developed a model based on Monod type relationships which simulates the utilization of organic substrates by bacteria in a biofilm. This model is based on steady state assumptions, and holds that a single substrate cannot be reduced below some theoretical minimum concentration that is required for microbial maintenance. In other words, if the substrate were present in limited amounts, it would be depleted first and growth would cease. According to Rittman and McCarty, the steady state biofilm concept is applicable to the groundwater environment, in which low substrate concentration limits biomass growth potential. This model is mathematically expressed as follows:

$$S_{min} = K_s \cdot \frac{b}{yk-b}$$

Where: S min = minimum substrate concentration for microbial maintenance

Ks = Monod half maximum rate concentration

b = first order decay coefficient

y = cell growth yield

k = Maximum specific rate of substrate utilization

A steady state biofilm by definition undergoes no net decay or growth for the whole biofilm. The limiting value (Smin) that is obtained through this process is based on steady state microbial utilization. Laboratory experiments performed by Rittman and McCarty have confirmed the existence of Smin and verified substrate utilization rates as predicted by the model.

Application of this steady state model to groundwater implies that residual contamination should remain in the water and that biodegradation will not occur at values less than Smin. Values for Smin are typically in the range of 0.1 to 1.0 mg/l (Bitton and Genoa, 1984). Monitoring of trace organics in petroleum contaminated groundwater does not support this, and in fact can be significantly lower. It is believed that a phenomenon known as secondary utilization is responsible for these observations.

In secondary utilization, a substrate with a concentration less than its Smin (secondary substrate) may be utilized provided a primary substrate is present in concentrations greater than its Smin (Rittman and McCarty, 1984). The primary substrate may be a single compound, an aggregate of many compounds, or composed of many secondary substrates. A secondary substrate need not share enzymatic pathways with the primary substrate, but the bacteria must be capable of transforming both.

A model developed by Rittman and McCarty in 1981 simulates secondary substrate flux into the biofilm if biofilm mass and rate parameters are known. It is mathematically expressed as:

$$\frac{dSf}{dt} = -\frac{KXf}{Ks} \cdot Sf$$

Where: Sf = rate limiting substrate concentration

t = time

Xf = active cell density

Ks = Monod half maximum rate concentration

K = Maximum specific rate of substrate utilization

Laboratory and field studies have demonstrated the concept of secondary utilization. In studies conducted by Bouwer and McCarty (1984), they showed that acetate could be removed to below its Smin concentration in a biofilm reactor. Additional field work in California reported by Roberts and McCarty (1980), provided evidence of secondary utilization of naphthalene, which was nearly completely degraded following the formation of an active biofilm in the subsurface.

E. Environmental Factors Affecting Biodegradation

Environmental factors which influence the maintenance and growth of microorganisms in the subsurface include: nutrients (nitrogen, phosphorous), oxygen, as well as temperature and pH. In the early studies of gasoline degradation in groundwater, it was theorized that large scale cleanups could be enhanced by the use of nutrient additions. Macronutrients such as nitrogen and phosphorous, as well as oxygen, were therefore evaluated as to whether they could be rate limiting in the biotransformation process. The first such laboratory studies were conducted by Raymond in 1975 in which it was found that supplements of air, inorganic nitrogen, and phosphate salts could increase the numbers of hydrocarbon degrading bacteria one thousand fold.

Following these initial investigations, a small scale field study was conducted by Jamison (1975). This study demonstrated that the addition of nutrients enhanced the growth in similar proportions to Raymonds lab studies. Jamison used varying nutrient formulations consisting of: sodium nitrate ($NaNo_3$), phosphate (PO_4^{-3}), magnesium sulfate ($MgSO_4$) and, ammonium nitrate (NH_4NO_3). Results from this study indicated that the form of nitrogen and phosphorous was not critical, however, improved results were obtained using a 0.1% (1000 ppm) solution each of NH_4NO_3, PO_4^{-3}, and $MgSO_4$. It was further concluded that in this aquifer, inorganic nutrients such as calcium and iron were not needed.

Raymond (1976) initiated a full scale bioremediation effort at the Ambler, Pennsylvania site using ammonium sulfate ($NH_4 SO_4$), disodium phosphate (Na_2PO_4), and monosodium phosphate ($NaPO_4$). Air was injected into the wells using down well diffusers. Concentrations of 30 percent nutrient solutions were injected by batch addition. Biodegradation of 1 liter of gasoline was estimated to require 44 grams of nitrogen, 22 grams of phosphorous, and 730 grams of oxygen.

Raymond reported results from additional work in California showing enhanced bioremediation of gasoline utilizing a mixture of carbonate (CO_3), hydrogen peroxide (H_2O_2), NH_3, PO_4, and calcium (Ca). Although concentration of all nutrients were not given, oxygen concentration (as H_2O_2) was maintained at 250 ppm. Brenoel and Brown (1985) investigated an industrial site in the midwest in which adsorbed hydrocarbon concentrations in soil ranged from 1000 - 3000 ppm.

Nitrogen and phosphorous in this project were added by batches as ammonium chloride and sodium phosphates so that levels were maintained at 100 - 300 ppm. Oxygen levels in the aquifer were maintained at 10 ppm, but no inlet concentration of H_2O_2 was given.

In 1985, Britton, of the Texas Research Institute, performed a feasibility study on the use of hydrogen peroxide to enhance microbial degradation. This grew out of the belief that the solubility of oxygen in water was limiting substrate utilization. Microbial tolerance to H_2O_2 was tested using sand columns.

Results from this test indicated that hydrocarbon degraders could tolerate up to 0.05% H_2O_2 (500 ppm), and by increasing H_2O_2 concentration incrementally, this could be increased to 0.2% (2000 ppm). In all instances, oxygen increased the numbers of microorganisms present, and was found to be the most important growth limiting factor.

Hydrogen peroxide can increase dissolved oxygen in groundwater to between 250 and 500 ppm (EPA, 1988, from Raymond, 1987). Additionally, the use of hydrogen peroxide requires less equipment, as it is added in-line along with the nutrient solution. Thomas and Ward (1989) caution, however, that the oxidant can be toxic to microorganisms at concentrations as low as 100 ppm. They further assert that toxicity problems can be minimized by starting with concentrations of 50 ppm, and then increasing in a step wise fashion to levels as high as 1000 ppm. Wilson _et_ _al_. (1986) note that the rate of hydrogen peroxide decomposition must also be controlled. Because hydrogen peroxide decomposes rapidly in the presence of iron, air bubble formation can occur, resulting in gas blockage and loss of permeability. To avoid this, they recommend the addition of phosphate to recirculated water to precipitate the iron.

As stated, the rate at which oxygen can be brought into contact with the subsurface microorganisms is a rate limiting factor in the remediation process. Table 3 was developed by Wilson and Ward (1986) to provide a comparison of the quantity of water (in pore volumes) needed to be exchanged with soil material in order to renovate residually saturated soils. These values were based on an

Table 3. Estimated Volumes of Water Required to Renovate Hydrocarbon Residually Saturated Soils (Wilson, 1986)

	Proportion Occupied By			
Soil Texture	Hydrocarbon	Air	Water	Pore Volumes
Stone to Coarse Gravel	0.005	0.4	0.4	5,000
Gravel to Coarse Sand	0.008	0.3	0.4	8,000
Coarse to Medium Sand	0.015	0.2	0.4	15,000
Medium to Fine Sand	0.025	0.2	0.4	25,000
Fine Sand to Silt	0.040	0.2	0.5	32,000

oxygen content of water at 10 mg/L. Based on these findings, it is clear that vast quantities of water are required to meet the oxygen demand of the hydrocarbons. Specifically, as many as 15,000 pore volumes in a medium to coarse sand.

Temperature and pH have long been known to be a factor in microbial growth due to the chemical reactions associated with the growth process. Considering temperature, the effects on growth are considered to follow a normalized bell shaped pattern, having some optimum temperature at the apex of the curve. Based upon the temperature range in which the microorganisms thrive, they are classified as psychrophilic (-5°c-20°c), mesophilic (20°c-45°c) or thermophilic (40°c-75°c). Some microorganisms are capable of operating across two classifications and they are termed faculatative. The majority of the hydrocarbon degraders fall in the 30°c - 40°c range and, therefore, are mesophiles or thermophiles (EPA, 1988 from Bossert and Bartha, 1984). Degradation of petroleum has been reported outside these values, however, and optimum values for microbial degradation given by Atlas (1981), are above 20°c and below 40°c.

The effects of pH on microbial growth is similar to that observed with temperature. That is to say that microbial growth response to pH follows a normalized bell shaped pattern, with optimum growth occurring again at the curve's apex. This effect is attributed to a change in enzyme activity as pH rises or falls about this optimum. For most bacteria, the pH extremes for growth occur between 4 and 9, with the optimum being somewhere between 6.5 - 7.5 (Benefield and Randall, 1988). According to Atlas (1987), most bacteria have limited tolerance for acidic conditions. Consequently, this will determine what organisms participate in the hydrocarbon degradation, unless pH is modified. Atlas also asserts that the overall rate of degradation is higher under slightly alkaline conditions.

IV. In Situ Bioremediation System Design

A. Introduction

Remediation techniques that have evolved for the cleanup of gasoline contaminated groundwaters can be summarized as one of three general techniques:

- groundwater pump and treat systems utilizing vapor stripping to remove volatile organics
- groundwater pump and treat systems utilizing granular activated carbon adsorption to remove gasoline constituents
- groundwater pump and treat systems utilizing bacteria to transform the gasoline constituents to harmless constituents

The biostimulation method which has been most developed, and is most feasible according to EPA (1987), is one that relies on aerobic, naturally occurring microbial processes. This method involves the optimization of environmental conditions by providing oxygen and nutrients through one of two types of delivery systems: an infiltration gallery system, or an injection well system.

The groundwater bioremediation design sequence that is generally followed begins with an investigation of the subsurface to determine the scope of the problem. Following a complete investigation of the subsurface hydrology, a microbial degradation enhancement study is conducted to determine degradability and optimum nutrient formulations. Following this, operation and monitoring of the system can begin.

B. Hydrologic Considerations

In designing an _in situ_ bioremediation system, controlling groundwater flow is critical to moving required oxygen and nutrients to the contamination zone and keeping contaminants and any injected water within the site boundary. In the groundwater control phase of the project, monitoring wells are installed in a predetermined pattern to provide data on water table gradients and groundwater flow directions, as well as contaminant concentrations. Soil borings which are extracted from the wells are analyzed to give an indication of the nature of the soil contamination and the site geology.

The location of monitoring wells is initially established based on general monitoring information found in existing U.S. Geological Survey maps, and knowledge of existing hydrogeological properties and well extraction activities in the area. The initial soil boring and monitoring well installation program is simply designed to surround the tank area, and provide upgradient and downgradient values of contaminant concentrations.

Water table elevation determinations and groundwater contaminant values are used to develop a map of groundwater flow direction and estimated gasoline plume dimensions. Actual maps from a site in Washington, D.C. are attached (Figures 5 and 6) showing groundwater contours and plume configuration. Once plume dimensions have been estimated, a general conceptualized cleanup approach can be postulated, however, design of the system cannot begin until further definitions of aquifer characteristics are obtained.

The hydrologic properties of interest in the aquifer are generally obtained through the performance of an aquifer pumping test. These properties consist of the aquifer transmissivity, the storage coefficient, the hydraulic conductivity, and the radius of influence. These terms are defined as follows:

<u>Transmissivity</u> - the rate at which water flows through a vertical strip of an aquifer 1 foot or 1 meter wide and extending through the full saturated thickness, expressed as gpd/ft of thickness.

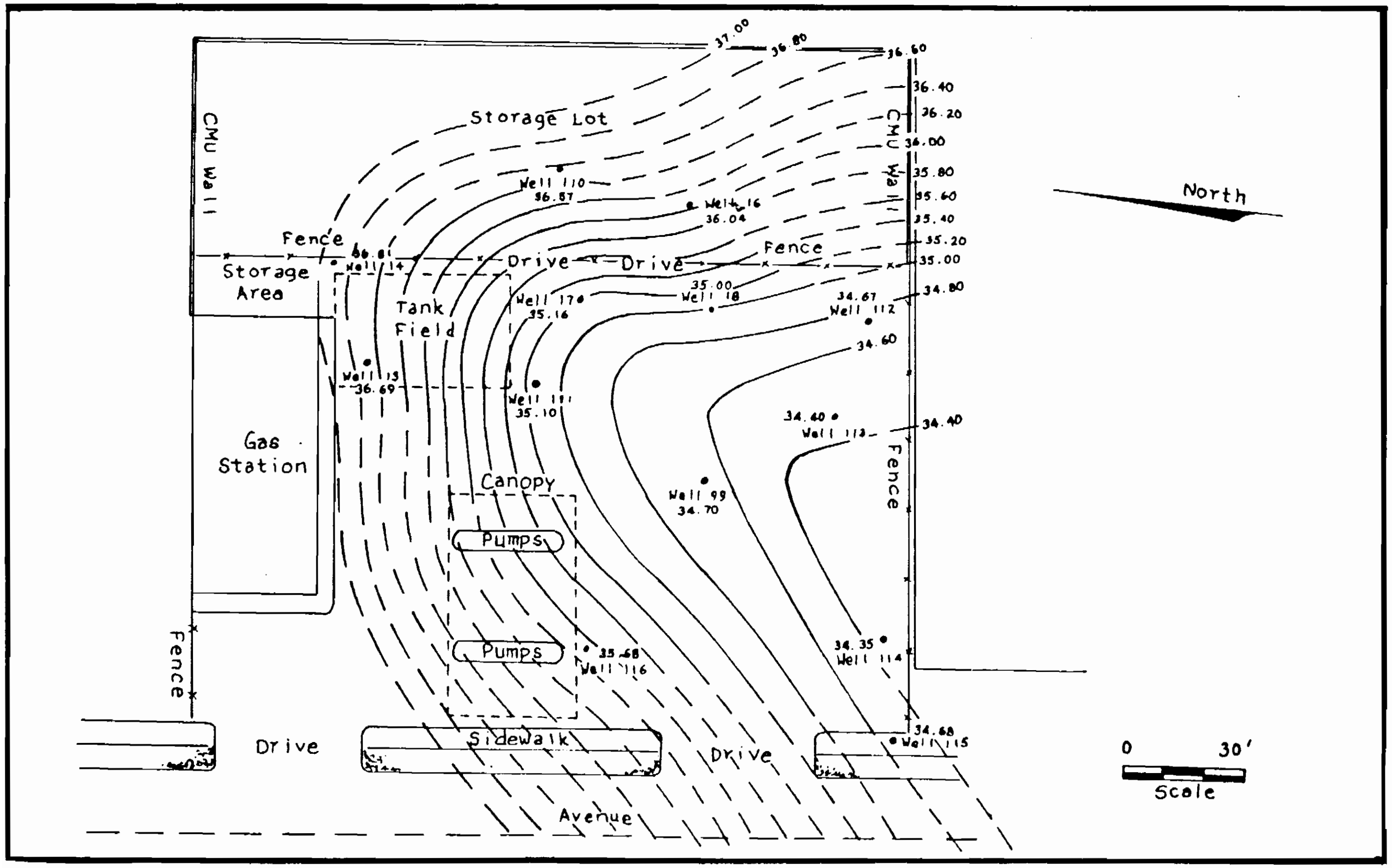


Figure 5 Contoured Water Elevation Map at Washington, D.C. Site

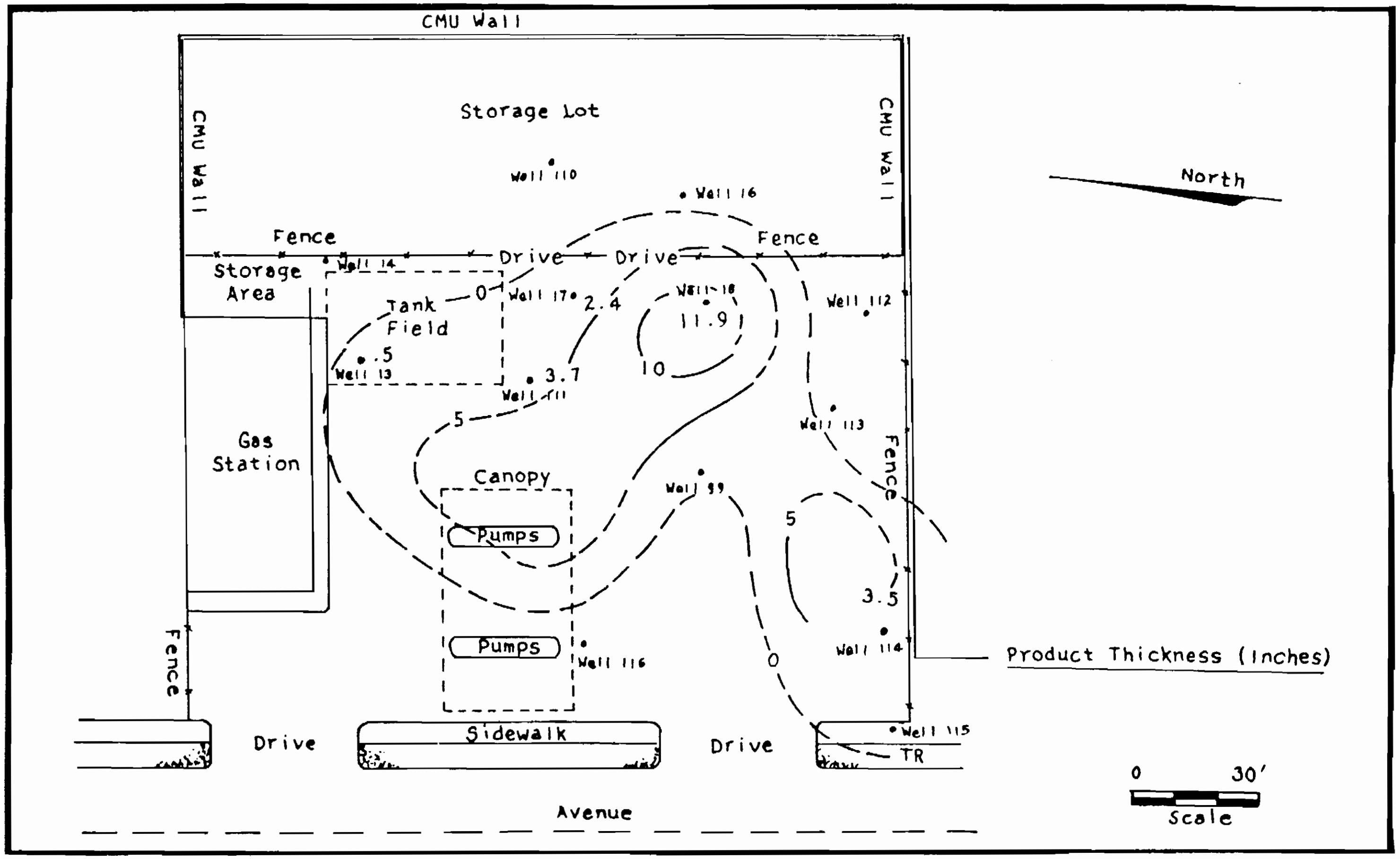


Figure 6 Product Thickness Contour Map at Washington, D.C. Site

*Coefficient of Storage - the volume of water released from storage per unit of aquifer storage area, per unit change in hydraulic head. In unconfined aquifers as discussed here, this is also called the Specific Yield.

*Hydraulic Conductivity - the capacity of a porous medium to transmit water.

*Radius of Influence - the horizontal distance from the center of a well to the outside limit of the cone of depression formed by pumping.

Measurements required for the pump test include; the static water levels just before the test is started, the elapsed time, the pumping rate, and dynamic water levels at various pumping intervals. Measurements of water levels after the pump stops are most important in verifying the aquifer coefficients calculated during the pumping phase (EPA, 1987).

These tests are performed by pumping water from a well and observing the resulting drawdown in the pumped well, or in a nearby well. It is desirable in aquifer tests to have at least three observations wells located at different distances from the pumping well. In reasonably homogeneous aquifers, this method can be used to determine the parameters listed above.

Utilizing mathematical relationships derived from Theis (1935), a graphical method of calculating aquifer coefficients and predicting drawdown of the aquifer is performed by plotting drawdown and time data taken during a pumping test (EPA, 1987). This data is plotted on semilogarithmic paper (Figure 7).

The coefficient of transmissivity is calculated from the pumping rate and the slope of the time-drawdown graph. The coefficient of storage is calculated by using the zero-drawdown intercept of the line and the distance from the pumped well to the observation well. These have been mathematically expressed as follows (EPA, 1987, from Cooper and Jacob, 1946):

(see page 71)

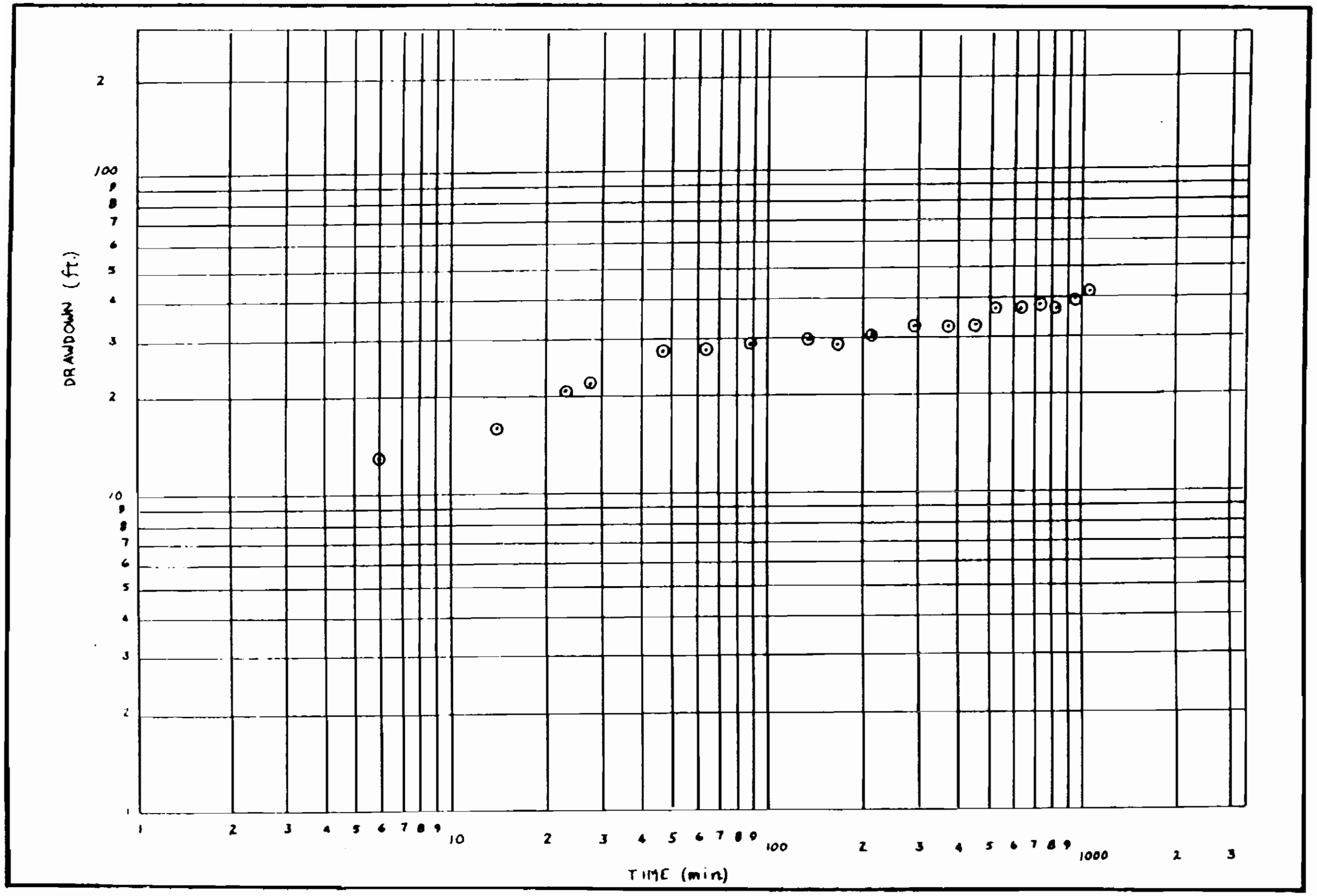


Figure 7 Time- Drawdown Well Pumping Test Graph

$$T = \frac{35Q}{s}$$

Where: T = coefficient of transmissivity, (ft^2/day)

Q = pumping rate, (gpm)

S = drawdown across one log cycle, (ft)

and: $S = \frac{Tto}{640r^2}$

Where: S = storage coefficient

t_o = intercept of the straight line at zero drawdown, (minutes)

r = distance from the pumped well to the observation well, (ft)

In addition to its use for calculating the aquifer constants, the time-drawdown diagram provides a graphical means of predicting future drawdown. The straight line in the graph may be extended to the right to indicate the drawdown that would occur in the observation well at the same distance from the pumped well after any period of continuous pumping. Once the slope of the time-drawdown graph has been established from a short-term pumping test, the straight line can be extrapolated to find the expected drawdown after longer periods of pumping at the same rate.

Utilizing the information obtained, the gasoline recovery system design can then proceed. The recommended pumping rate to obtain a desired radius of influence can be calculated utilizing an equation relating parameters measured during the pumping test and geological investigation as follows (Keely, 1983):

$$r = \frac{Q}{H.2\pi.O_e.Vnat}$$

Where: Q = average discharge, (gpm/ft)

H = available drawdown, (ft)

r = radius of influence, (ft)

$Vnat$ = Darcian flow velocity, (ft/day)

O_e = estimated porosity

Once all of the above groundwork has been performed, the well spacing on the site is established to ensure adequate overlapping of the cones of depression.

Values calculated for radius of influence are usually adjusted downward so greater overlap occurs. Additional modifications are usually necessary following system start up due to inherent inaccuracies in the mathematical models used, and due to non-homogeneous conditions found within the aquifer.

C. Biological and Geochemical Considerations

In the microbiological phase of the design process, aseptically collected soil samples are first analyzed for total numbers of bacteria able to grow on hydrocarbons as opposed to a general microbiological medium. This data is used to demonstrate the presence of hydrocarbon consuming bacteria to total bacteria counts. This ratio is used to determine the level of microbial adaptation or selection which has occurred naturally.

Site groundwater is then evaluated for various cation and anion components, as well as dissolved oxygen, pH, phosphate, and ammonia concentrations. Additionally, groundwater is collected to screen for the nutrient mixture which will provide for optimal bacterial growth. Soils are then screened for their phosphate adsorping characteristics and the effects on hydrogen peroxide stability through the use of permeameter studies.

Nutrient requirements are generally taken to be site specific and, therefore, laboratory studies must first be conducted to determine groundwater phosphate and nutrient concentrations. These are followed by studies to determine optimum nutrient formulations. While many of the industry nutrient formulations are proprietary, Brenoel and Brown (1985) have suggested that concentrations of nitrogen and phosphorous in the 125 - 300 ppm range be used. The form of the nutrient can also be important. In a study conducted by Raymond and Minugh et al. (1983), ammonium sulfate was found to be more efficient than ammonium nitrate in microbial growth.

The availability of these nutrients to the microbes is optimum at, or ranging about neutral pH values, and depending on the characteristics of the soil, the nutrients can be removed from solution by sorption onto the soil. In a study conducted by Brubaker and Crockett in 1986, about 90 percent of the ammonium and phosphate, and 70 percent of the hydrogen peroxide added to a sandy soil with low calcium, magnesium, and iron was recovered (EPA, 1987). They concluded that this soil type had low adsorptive capabilities. Groundwater should therefore be monitored continuously to evaluate treatment effectiveness, as breakthrough of these parameters could indicate contaminant removal in that area.

D. Bioremediation System Configuration

As shown, there are many potential limiting factors for the in situ remediation of gasoline contaminated groundwater, but none is so critical as getting the treatment process to the contaminated material. The engineering design of groundwater collection, recovery, and recirculation systems is highly site specific, however, two general system types have emerged. The first is an infiltration gallery system, which is useful in shallow aquifer applications, and the second is a well injection system, applicable to deeper aquifers. Typically, both are operated as closed looped systems with recycling of aquifer water to maintain the nutrient and microbial levels, as well as control of the water flow (EPA, 1988).

Figure 8 shows a typical design of an infiltration gallery remediation system. The principle features of this design include; an infiltration gallery, a system of recovery wells, a nutrient addition tank, a hydrogen peroxide tank, and a system of monitoring wells. Each of these is discussed independently as follows.

The infiltration gallery configuration can be varied, however, it is generally designed as either a system of trenches, or a large open excavation which is fitted with a slotted pipe distribution system and backfilled with drainage rocks. The gallery location is selected based on areas identified as having high concentration and on groundwater flow direction. This may be accomplished by injecting into the shallow subsurface within the source area in such a way that the oxygen and nutrients follow the same path as the contaminants. The gallery additionally is excavated only to an elevation above the source so that the water added can seep into the ground and make contact with the capillary zone and other affected soils.

The critical factor determining the size and number of galleries is the rate of water infiltration, which is calculated through the previous hydrogeologic investigation. The design and placement of recovery wells is also a result of the hydrogeologic investigation and is based upon the calculated aquifer pumping rates and radius of influence of

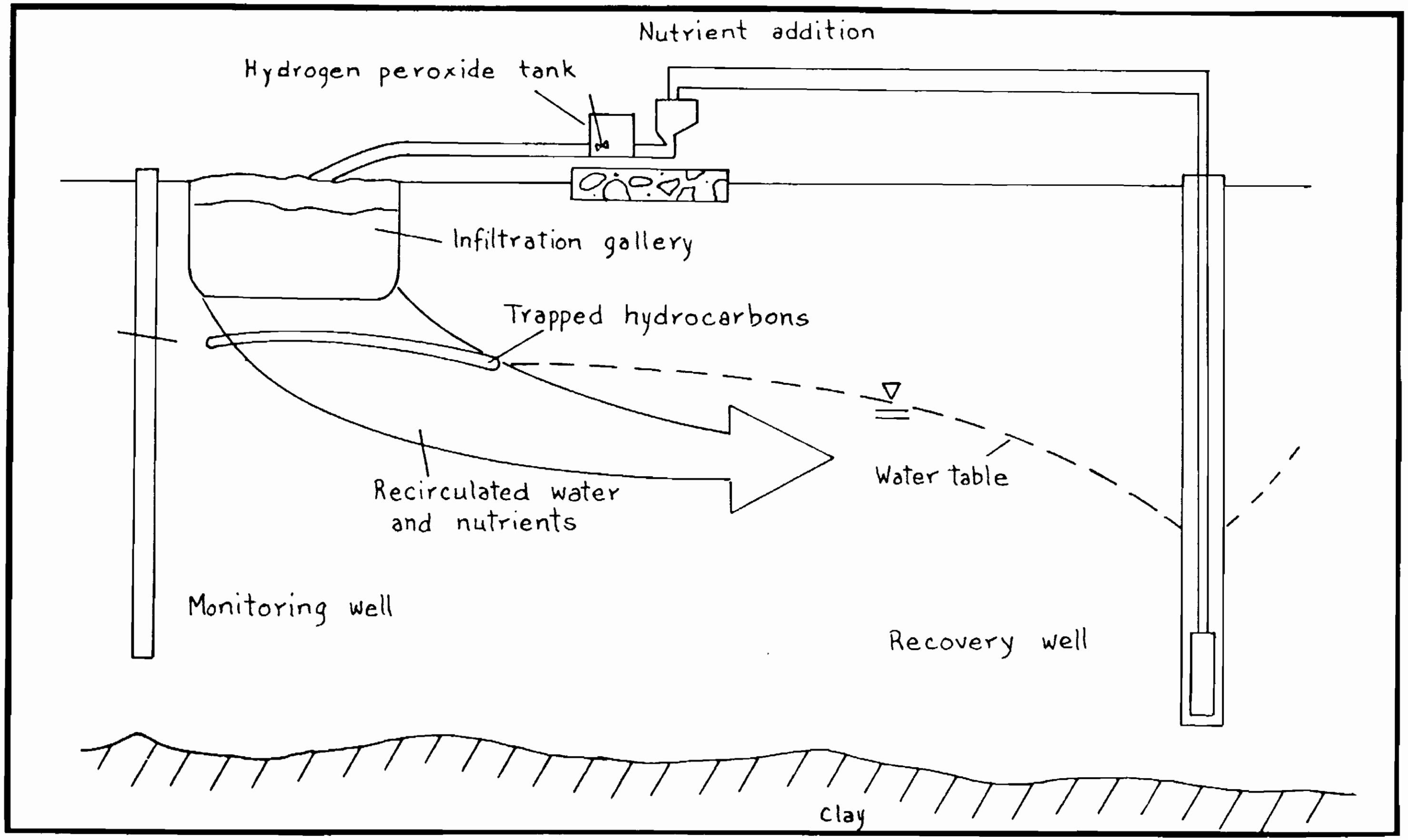


Figure 8 Typical Infiltration Gallery Bioremediation System Design

the recovery well(s). The radius of influence is the parameter which directly impacts well spacing, pumping rates, and pumping cycles. The maximum efficiency for well operation in an unconfined aquifer occurs at approximately 67 percent of maximum well drawdown and, therefore, pumping rates and radius of influences are usually adjusted downward accordingly (EPA, 1987). Wells are spaced such that adjacent radii of influence overlap and contaminant plume coverage is complete.

The nutrient addition tank is actually a mixing tank where nitrogen, phosphorous, and trace elements are added to pumped groundwater for reinjection into the infiltration gallery. The hydrogen peroxide tank is the storage unit for the peroxide. In cases where free floating gasoline is suspected to interfere with the process, these tanks would be preempted by an oil-water separator tank. The nutrient tank is independent of the hydrogen peroxide tank to provide for system flexibility. Specifically, the nutrients are generally added first, followed by the hydrogen peroxide. This is because simultaneous addition of these could result in excessive microbial growth around the injection point, which could plug the formation.

Monitoring wells are critical to evaluating the performance of the bioremediation as well as to monitor upgradient and downgradient contaminant concentrations. The wells are generally constructed of four inch slotted polyvinyl chloride (PVC) pipe and installed to a depth which takes into consideration any groundwater table fluctuations. They can be fitted with a number of different pump types currently on the market. For pumping applications less than twenty two feet, suction lift pumps are used. For deeper aquifer applications, submersible pumps or downhole turbine pumped wells are used.

Figure 9 shows a typical design of an injection well system. The major difference between infiltration gallery and injection well system design is the mode of transfer for the injection/recycled water. Utilizing injection wells, while more costly, provides some advantages to site remediation over infiltration gallery techniques. Injection may provide ways of contacting soil contamination in areas that are unsaturated under ambient hydrologic conditions. Also, where the direction of groundwater flow varies over the course of the year, injection wells can be utilized to provide more flexibility in the treatment process by varying the injection and recovery points.

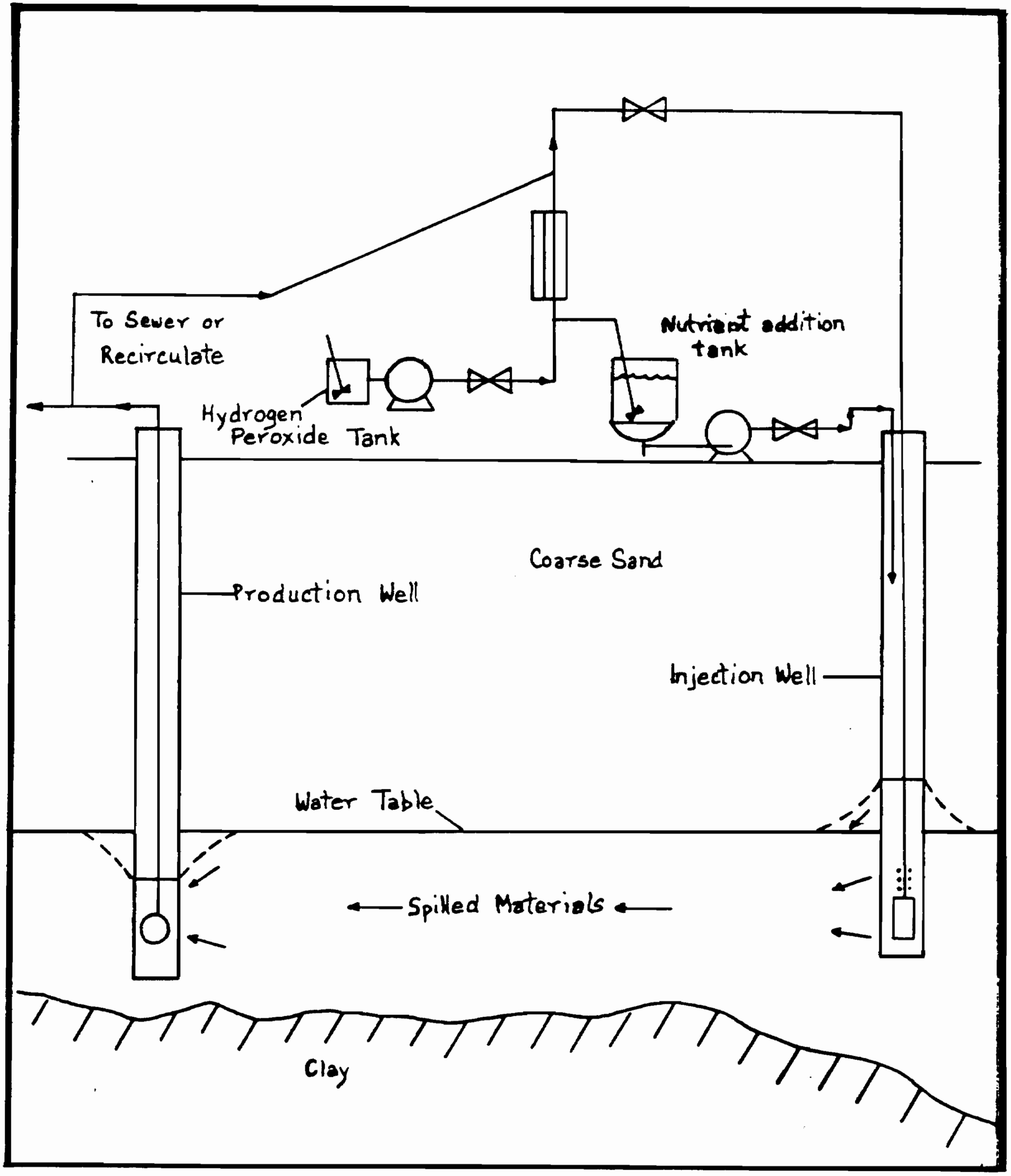


Figure 9 Typical Injection/Recovery Well Bioremediation System Design

The critical parameters needed in the design of an injection system include the injection rate(s) and the distance between the injection points. This is determined through the hydrogeologic investigation.

Injection rates must be sufficient to create the desired aquifer "mounding", such that the hydraulic head is increased between the injection point and recovery point. This in turn will determine the radius of influence of the injection and, thus, the number of injection wells. Under ideal conditions, in-situ bioremediation projects are designed so that the time from injection to recovery is less than four weeks (Westray and Brubaker, 1988). This minimizes the effect of nutrient adsorption to the soil matrix and peroxide decomposition. It further provides a convenient time frame to conduct monitoring of the process to changes in injection and recovery rates and conditions.

The performance of both the gallery and injection well systems, and the proper distribution of the peroxide and nutrients, can be monitored by measuring the parameters found in Table 4. As noted, many of the inorganic analyses only need be conducted initially to establish baseline concentrations. Additional analyses would then be conducted on an as-needed basis.

Utilizing the baseline values, the results of soil microcosm tests will determine macro and micronutrient requirements, and determine any pH control, if needed. In general, samples should be drawn on a monthly basis (for a minimum of one quarter) to optimize the nutrient and oxygen delivery process, and verify system parameters.

Cation analysis is conducted initially to predict potential problems with the precipitation of metal oxides and hydroxides. Hydrocarbon degrading bacterial counts are performed to verify bacterial concentration increases and decreases as a function of substrate availability. Carbon dioxide and dissolved oxygen are used as indicators of microbial activity in the aquifer. Ammonia and phosphorous concentrations are analyzed continuously along with total petroleum hydrocarbons to monitor microbial utilization of nutrients and substrate.

Table 4. Recommended Laboratory Analyses for Groundwater Bioremediation Projects

Organic Parameters	Inorganic Parameters	Bacterial Parameters
Benzene *	Ammonia (NH_3)	Total CFU/gm *
Toluene *	Phosphate (PO_4)	Hydrocarbon
Xylene *	Calcium (Ca) *	CFU/gm
Ethylbenzene *	Magnesium (Mg) *	
Carbonate (CO_3) *	pH	
Total Petroleum	eh *	
Hydrocarbons (TPH) (CO_2)	Manganese (Mn) * Iron (Fe) * Chlorides * Dissolved Oxygen (D.O.)	Carbon Dioxide

Note: * = analyze initially and then as-needed

CFU/gm = colony forming units/gram

Following this, sampling should be scaled back to a quarterly basis and analyses should be limited to those parameters which are fundamental to process operation. These include: CO_2, TPH, NH_3, PO_4, pH, and dissolved oxygen.

SECTION - FIVE DISCUSSION

A. Subsurface Physical and Chemical Factors Affecting Remediation

The physical and chemical processes which act to impede remediation of gasoline contaminated groundwater include adsorption, dispersion, and vapor storage. Adsorption of the gasoline to a soil particle is primarily a function of the chemicals sorption potential, and the organic content of the soil. Even when organic contents are small, however, the chemical constituents found in gasoline generally have high adsorption coefficients, ranging from 190 - 88,000. Additionally, gasoline contains many constituents with high water solubilities, and spills historically result in prolonged contact time between release and remediation.

The resultant adsorption of the gasoline constituents is believed to play a significant role in the observed rebounding of chemical concentrations following conventional pump and treat remedial techniques. Researchers Dragun and Keely have postulated that the desorption process which takes place as a result of falling contaminant concentrations following remediation is not initially rate limiting. As the remediation process continues, however, and equilibrium concentrations are reached, rates of desorption slow down markedly (Figure 3). This phenomena has been termed retardation.

From a regulator's perspective, this can lead to premature halting of remediation. Recognition of this has resulted in improved pump and treat methodology, as well as biorestoration techniques. One of the promising innovations in the area of classic pump and treat technology is the idea of intermittent operation of remediation wellfields, termed pulsed pumping.

Pulsed operation of hydraulic systems is the cycling of extraction or injection wells on and off in active and resting phases. The resting phase of pulsed-pumping operations can allow sufficient time for equilibrium concentrations to be reached in local ground water. Subsequent to each resting phase, the active phase of the pulsed-pumping cycle removes the minimum volume of contaminated groundwater at the maximum possible concentrations, allowing for the most efficient treatment. Considering vapor storage, the primary mechanisms of vapor transport in the underground tank environment are ordinary gaseous diffusion and advection. The porosity of the media is critical in determining the rate of this process. If a leak is small and there is no great pressure buildup, vapor transport of the volatile contents will occur through ordinary gaseous diffusion. The concentration gradient which is established between the leaked volatile product and uncontaminated soil air results in the movement of the leaked vapor usually in all directions into the soil. Lyman has reported diffusion rates of hydrocarbons in air at 0.06-0.09 cm^2/s.

Remediation techniques for handling gasoline released from an underground tank have mostly centered on removing free floating and dissolved constituents by installing wells to depress the level of the water table and removing the dissolved gasoline from the groundwater by means of some adsorption or stripping technology. This represents only a partial solution to the recovery problem since the majority of gasoline is usually contained in the unsaturated zone above the water table.

As the vadose zone does not have the hydraulic transfer system available in the saturated zone, in order to remove this gasoline, in-situ soil venting techniques have been developed. A soil venting process involves drawing air through the soil to evaporate the gasoline, passing the air through activated carbon beds to adsorb the volatile organics, and then finally recharging the carbon beds in the field by means of steam to produce pure gasoline for proper disposal.

The operation must be carried out above the water table, and the design and layout of well points or trenches for placing a vacuum on the ground depends on the ability to lower the water table, and on the openness of the soil. Where water tables are deep, in the range of 15 feet or more, and the soil is fairly open containing gravel or coarse sand, a strong vacuum can be placed on just one well point to draw air from a radius of up to 100 feet. The minimum ratio of air to degraded hydrocarbon has been reported by Raymond et al. (1990) as 13 pounds of air to one pound hydrocarbon. This is compared to 10,000 pounds of water to degrade one pound of hydrocarbon in a conventional pump and treat system.

The results of a vapor recovery technique at a site which was referenced by Conner, (1988), was that total volatile organic concentrations in the groundwater were reduced from

5,400 ppb to trace levels at one monitoring well where most of the venting had occurred, and from 6,400 ppb, to 4,600 ppb at a second monitoring well where less venting had occurred. Since no treatment of the groundwater occurred, reduced concentrations of gasoline in the groundwater must have resulted from evaporation of gasoline from its surface. In a separate study conducted by Dey _et_ _al_. (1991), it was estimated that 85 pounds/day of gasoline could be recovered by 4 extraction wells on a site in New Jersey.

An obvious additional benefit of soil venting is that it introduces oxygen into the soil. Bacteria which are present in the unsaturated zone require a adequate supply of oxygen to accelerate the biotransformation process. By introducing oxygen into the soil through the soil venting process, decomposition of residual gasoline in the soil is increased substantially.

As noted, examination of data from UST gasoline spills has shown that the largest volume of gasoline is retained in the free floating and capillary zones. While these zones contain the largest fraction of the gasoline by volume, however, they impact a far less area than the dissolved phase of the gasoline / groundwater mixture. This is attributed to physical and chemical dispersion, whereby concentration gradients and natural groundwater flow rates allow the dissolved phase of the gasoline to be transported over large distances.

Groundwater flow rates are generally considered to be non-turbulent (cm/day - m/day) and, thus, dispersion would not normally be considered a significant environmental threat. This is not the case, as spill sites have generally gone unnoticed for many years, and allowed contaminant dispersion to become a factor in the area extent of contamination. As much as 80 percent of the total contaminant area in a site studied by Brenoel and Brown (1985).

The remediation of dissolved contaminants can be accomplished through conventional groundwater pump and treat techniques. These same techniques are used to recover free floating gasoline product. The process of designing a groundwater remediation system involves controlling the local hydraulic gradient through the establishment of what are termed "cones of depression". The hydrologic properties of interest are obtained through the performance of well pumping tests.

Utilizing the information obtained, the design pumping rate, and the hydrologic radius of influence can be calculated to capture the dissolved plume.

B. Hydrologic Factors Affecting Remediation

One of the primary problems associated with conventional pump and treat technology is that the design criteria for groundwater pumping systems rarely takes into consideration the impact of the remediation itself. With respect to the removal of gasoline from aquifers, the literature suggests three (3) major areas where design considerations do not always meet their objectives. These are: proper equipment selection, the number of wells and their placement, and pumping rate design.

Complex groundwater flow patterns, which are set up by oversimplified well placement, can result in certain parts of the aquifer being flushed well and others poorly. Keely (1989) asserts that this occurs because waters which are moving along the groundwater flowline move rapidly and proceed directly into the pumping well from upgradient, whereas the waters found at the boundaries of the capture zone move much more slowly. In addition to this, a possible result of incorrect overlapping well capture zones could be that previously uncontaminated portions of the aquifer at the outer boundary may become contaminated by the operation of an well that is located too close to the plume boundary, thereby drawing gasoline through the clean zone.

In addition to the problems stated above, using too few recovery wells can lead to the need for increased pumping rates, and subsequent "deep" cones of depression to control groundwater gradients. When this happens, the relatively pure gasoline found in the recovery well is drawn into the depression, thereby causing the area surrounding the well casing, that has been previously dewatered, to trap residual gasoline below the water table. This trapped gasoline can become a continual source of contamination which will persist by nature of its adsorptive ability and wetting properties when the recovery well is turned off.

Pumping equipment used in the recovery of gasoline can vary significantly. There are essentially two major types of pumps: single, and dual pump systems, however, there is considerable variation within these categories (pneumatic, electric, etc.) In a single pump system, both gasoline and water are recovered through a single pipeline to aboveground storage tanks or oil/water separators. Two problems are associated with single pump systems: 1) large volumes of contaminated water must be stored, treated, and disposed of; and 2) during pumping, gasoline and water are mixed, thereby complicating aboveground separation. For these reasons, single pump systems are most commonly employed for smaller spills when the gasoline-water recovery rates are relatively low.

Dual pump systems are favorable to single pump systems, and operate in the following way. Initially, the water table depression pump probe is set at the depth in the well to which the water table will be depressed. The water table depression pump is then lowered approximately ten feet beyond the probe and pumping is begun. As water is pumped out of the well, the water table and floating product are drawn down until the water pump probe detects the presence of hydrocarbons. When this occurs, the water pump will cease pumping and the depressed water table will rise slightly. As soon as the water pump probe detects water again, however, it will resume pumping and the depression will be maintained.

Once a constant depression has been established, the product pump is deployed. The pump inlet and the probe of the product pump are set at the same depth, a few inches above the probe of the water table depression pump. As the water pump draws in groundwater, gasoline will accumulate in the depression until the hydrocarbons are detected by the product pump probe.

In addition to limiting the amount of water that must be treated, the dual pump system has another advantage over the single pump system in that the dual pump functions automatically. Water table depression and product removal are constant, and the system can operate for weeks or months with only periodic inspections. Once the gasoline plume has been drawn down to within a fraction of an inch, the product pump probe will no longer be able to detect the remaining gasoline. At this point, the product pump turns off, and the water table depression pump is elevated to the depression and allowed to pump the mixture of water and the remaining gasoline out of the well.

Other recent advances in groundwater pump and treat recovery includes the use of an adaptation of shallow well-point dewatering systems commonly used in the construction industry (Hayes, 1989). This system consists of multiple wells or drive points which are manifolded to a large capacity suction lift (vacuum assisted) pump. The wellhead on each recovery well is sealed to create a vacuum within the well casing. Generation of the vacuum increases the effective difference between pressures inside the well and the adjacent aquifer formation.

With the use of this system, there is an additional simulated drawdown in the well which enhances the flow. Additionally, the use of the multiple - shallow well system reduces the possibility of creating deep cones of depression, thereby eliminating the problems of contaminant smearing associated with this. Other advantages to suction lift systems include the ability to pump over a wide range of rates, and the ability to be pumped dry without harm to the pump.

C. Biodegradation Fundamentals

Microbial degradation of gasoline has been observed to occur by aerobic respiration, anaerobic respiration, and fermentation. These methods differ primarily in their utilization of external electron acceptors in the respiration process, the types of electron acceptors used, and in the energy yielded from the transformation. Enzymes control the biotransformation process and thus the critical reactions which yield energy for biomass synthesis.

Evidence that microbial populations exist in contaminated, as well as uncontaminated aquifers, has been verified by various researchers including Wilson, Novak, Jamison and Ghiorse. The microbial forms of importance in the gasoline biotransformation process are bacteria. Specifically, it has been reported by Wilson (1983) that uncontaminated aquifers which are oligotrophic by definition, are populated with gram positive forms. In contrast to this, Jamison and Raymond (1975) reported that a gasoline contaminated aquifer under study in Ambler, Pennsylvania yielded considerably greater numbers of gram negative bacteria, although gram positive forms were present. Further documentation of this has been provided by Smith (1985), in a study of a creosote contaminated site.

The gram negative bacterial organisms of significance to the biotransformation process include Pseudomonas, Acinetobacter, and, Flavobacterium. The gram positive forms most often cited are Nocardia and Micrococcus. It is probably safe to say that degradation occurs through the action of mixed cultures, with a predominance of some organisms due to growth rates. The aerobic respiration process utilizes oxygen as the external electron acceptor. In doing so, water is formed.

Aerobic respiration yields greater energy returns than the other processes. Microorganisms recover this energy for maintenance and synthesis of iomass through substrate level phosphorylation and oxidative phosphorylation, where adenine diphosphate is converted to adenine triphosphate. The overall effect of these energy recovery processes is a high rate of contaminant assimilation to the biomass. In the context of groundwater remediation, this means increased substrate utilization and, therefore, more rapid remediation.

Evidence of increased remediation rates using aerobic systems has been shown by various researchers and successful case histories continue to dominate the remediation literature. Even so, it has been shown that alternate electron acceptors can and are utilized in the biodegradation process (anaerobic respiration). This is probably the result of having mixed bacterial cultures with some bacterial forms preferentially being denitrifiers.

The anaerobic respiration process involves the use of alternate electron acceptors in the systems energy recovery processes. The most common alternate electron forms in the natural order of their use are; nitrate, manganese, iron, sulfate, and carbon dioxide. Metabolic pathways are considered to be the same as those found in the aerobic process. The major difference between these two processes is the energy yields. Because energy yields are lower in anaerobic systems, substrate utilization rates are lower, and thus contaminated assimilation is reduced.

In studies conducted to compare aerobic to anaerobic respiration rates (Wilson, 1986), aerobic conditions significantly reduced overall gasoline remediation timeframes from 8 weeks to 2 weeks. Given this, it remains to be determined whether the addition of nitrate as an alternate electron acceptor can further improve remediation rates in the presence of oxygenated aquifers. Britton (1989) reported favorably on this technique in the degradation of phenol. In contrast to this, Novak (1989) has reported that the addition of nitrate to soils already described as "slow", does not increase biodegradation rates. Fermentation is a respiration process which is characterized by a lack of external electron acceptors and, therefore, no utilization of an electron transport chain. Energy yields from the process are extremely small, and substrate utilization rates are also appropriately small.

Current theory found in the literature suggests that fermentation of gasoline organics does occur. It would appear, however, that in the degradation of aromatics, molecular oxygen must be present to attack the ring structure, as this is only hypothesized to occur through the use of a dioxygenase enzyme. Strict fermentation, therefore, probably plays any insignificant role in the biodegradation of gasoline, and this has been reported by Bartha (1986). In attempting to model the biotransformation of organics in a subsurface aquifer, a biofilm model has been postulated by various researchers (Wuhrman, Rittman and McCarty). Within the biofilm, two processes are theoretically occurring simultaneously; diffusion of the substrate into the biofilm, and utilization of the substrate by the bacteria. The flux of the substrate over the diffusion layer is taken to follow Ficks law, which states that diffusion is a function of the molecular diffusivity of the rate limiting substrate, and the concentration gradient across the biofilm surface.

A model for substrate utilization has been developed by Rittman and McCarty (1980) and is based on the theory of a minimum substrate concentration (Smin) for microbial maintenance. This model holds than Smin is a function of the Monod half maximum rate concentration, and an expression for substrate utilization which incorporates first order decay coefficients. Predictions of Smin utilizing this model yield a value range of 0.1 - 1.0 mg/L (Bitton and Genoa, 1984).

Many organic substances found in gasoline have been biodegraded to levels below this, (Roberts and McCarty, Bower and McCarty). A secondary substrate utilization mechanism has been theorized to be responsible for this (Rittman and McCarty, 1984). A model describing this process has also been developed by these researchers. is model holds that the concentration of the secondary substrate is a function of the maximum specific rate of substrate utilization, the active cell density, and the Monod half maximum rate concentration.

From a practical perspective, these models can be used to predict cleanup times if reasonably accurate estimates of mass loading in the subsurface can be calculated. Given this ability, bioremediation should attract an increased audience of responsible parties as they learn of its expedited remedial abilities. In addition to this, a more accurate prediction of costs associated with bioremediation should be achievable.

D. Bioremediation System Design Parameters

Prior to biological in-situ treatment system design, a number of site specific factors must be investigated. These include the hydrologic properties of the subsurface, as well as the biological and geochemical properties. The first step in the design process is to define the direction and rate of groundwater flow along with other hydrologic parameters. Following this, a laboratory study is conducted to determine the microbial treatability of the groundwater, and the optimum nutrient requirements for microbial growth. Nutrient requirements analysis is based on the geochemistry of the site, as well as results from studies aimed at optimizing biomass growth. The final treatment system design results from a integration of the data obtained from these two independent investigations.

The hydrologic phase of the program is designed to control the groundwater flow. This is accomplished through the manipulation of the water tables hydraulic gradient. An investigation of the subsurface hydrology utilizing well pumping tests gives the parameters necessary to design an appropriate groundwater control system. This system is fashioned so as to draw the contaminant plume into a series of recovery wells. This hydrologic information will also determine the configuration of the reinjection system. That is, whether an infiltration gallery, or injection wells are used. These are the prominent bioremediation system configurations used today.

Important hydrologic considerations in the design process are; the desired radius of influence on the recovery or injection wells, and the depth of the subsequent aquifer depressions. These parameters are related through the aquifers characteristics ability to store and transmit water. Design of a groundwater control system must include predetermined values for these as they relate to the aquifer characteristics.

The success of getting control of the subsurface hydrology is the most critical element in the design process. This is due to the reliance on this system to carry oxygen and other nutrients to the subsurface. The ability of the aquifer to produce water can, therefore, be key to this process. In extremely tight soils such as silts and clays, the hydraulic conductivity is extremely low, and transfer of the oxygen and nutrients would be limited. While this does not preclude these soil types from bioremediation, it suggests that alternative remediation techniques should be considered.

The greatest limiting factor in aerobic hydrocarbon degradation (short of the delivery system) is oxygen. As soils become saturated by either gasoline or water, anoxic conditions result by excluding oxygen from the soil pore spaces. Oxygen is critical to the aerobic degradation process as it serves as the terminal electron acceptor in microbial metabolism. Additionally, molecular oxygen is required as a cosubstrate for the enzyme responsible in the degradative pathway of saturated aliphatic and aromatic hydrocarbons.

The supply of dissolved oxygen may limit in-situ biotransformation processes. Depending upon the aquifer temperature, only 8-12 mg/L of dissolved oxygen can be achieved through sparging of the air into the aquifer. Because of this, alternative oxygen sources have been sought. The most promising alternative is the use of hydrogen peroxide.

As hydrogen peroxide decomposes, it forms one molecule of water and one half molecule of oxygen. Hydrogen peroxide decomposition is catalyzed by the presence of metal ions in the subsurface, and can be dissolved to concentrations of 500 parts per million (ppm) or higher, whereas oxygen concentrations are limited to 40-50 ppm (utilizing pure oxygen systems). Because of its improved solubility, hydrogen peroxide can vastly improve the efficiency of oxygen transfer to the microorganisms, however, it can also be toxic. Before application of hydrogen peroxide to the subsurface begins, the tolerance range of the microorganisms must be determined.

Macronutrients such as nitrogen and phosphorous have also been found to be potentially rate limiting in the biotransformation process. These nutrients are required as building blocks of cell protein, and as these are generally found in low concentrations in uncontaminated aquifers, they become limiting as the microbial population expands to degrade the organic contaminants. Because of this, these nutrients are added to the contaminated aquifer as a part of the system design. The quantities of these nutrients in the influent mixture are proprietary, as are the nutrient formulations. An indication of levels needed to be maintained, however, is given by a range of 100 - 300 ppm (Raymond, 1990).

Various nutrient formulations have been used by researchers to optimize gasoline utilization. The formulati
prescribed for each site appear to be
geologic matrix of interest
studies must be performed t
considerations in this proce
magnesium and calcium presenc
the aquifer through the applic
and plug the injection or recov

Iron can act as a catalyst in the decomposition of hydrogen peroxide. If decomposition of the peroxide is too rapid, bubble formation can occur and block the formation. This can be controlled with phosphate addition to the reinjected water. Additional consideration may be given to any micronutrients necessary for a healthy bioculture. An analysis of existing cations and anions in the groundwater will indicate this. Lastly, it is generally accepted that nutrient addition should precede hydrogen peroxide addition. This is due to the possibility of rapid biological film production at the injection point, and subsequent clogging of the aquifer.

SECTION SIX - SUMMARY AND CONCLUSIONS

The major objective of this report was to discuss the predominant aspects of fate, transport, and transformation of gasoline in subsurface aquifers as these factors relate to the failure of conventional groundwater pump and treat technology. An additional objective of this report was to identify the parameters necessary to conceptually design a groundwater bioremediation treatment system.

With regard to the transport of gasoline in the environment, the elements of bulk and advective fluid flow control the initial movement into the vadose and saturated zone. Following this, adsorption of the contaminant to the soil matrix; vapor transport of the gasoline through unsaturated pore spaces; and mechanical and chemical dispersion of the dissolved groundwater phase, all act to transport the gasoline over extensive areas.

The effects of advective fluid transport, as well as dissolved contaminant dispersion, can be adequately addressed using properly designed pump and treat technology. In contrast to this, bulk transport to subsurface capillary zones, vapor transport to unsaturated zones, and chemical adsorption of contaminants in the aquifer all pose difficult remediation questions, as they do not respond well to pump and treat technology.

Biotransformation processes such as aerobic and anaerobic respiration occur naturally in subsurface aquifers. This observation has lead to numerous successful applications of in situ microorganism assisted remediation of gasoline contaminated groundwater. The microorganisms responsible for the metabolism of gasoline organics are generally characterized as mixed bacterial biofilms. The predominant bacterial forms found are of the genus Pseudomonas and Nocardia, although others have been identified.

Several environmental factors are known to influence the ability of microorganisms to metabolize gasoline organics. These factors include, but are not limited to, temperature, pH, oxygen, and nutrient availability. In addition to this, the hydrologic properties of the subsurface are critical to efficient microbial utilization of the contaminant.

A commonly held theory of microbial metabolism is that microorganism population increases until limited by some metabolic requirement such as substrate or nutrients. A model based on biofilm kinetics has been employed to describe this. In addition to this, transport processes across the biofilm are limiting. A secondary substrate utilization theory has been formulated to explain lower than expected contaminant concentrations utilizing the biofilm model.

Aerobic bioremediation is currently defined as the most effective means of metabolizing gasoline in groundwater. Before this technique can be implemented, a thorough understanding of the subsurface hydrology, chemistry, and biology must be obtained and used to design the treatment system. Addition of oxygen via the breakdown of hydrogen peroxide, as well as the addition of nitrogen, phosphorous, and trace minerals is required to stimulate the microbial population.

Groundwater hydraulic patterns must be controlled to capture aquifer contaminants and distribute the microbial stimulants. Two systems have emerged in the remediation industry: infiltration galleries, and injection wells. Both of these systems rely on conventional groundwater pumping methodology. The difference is in their treatment distribution systems, and the desired area to be treated. These systems may be of limited usefulness in low permeability aquifers.

Alternative electron acceptors other than oxygen have been demonstrated to succeed in the metabolism of gasoline organics. The most prominent forms known include nitrates, inorganic minerals such as iron and manganese, sulfates, and carbon dioxide. It has not been established that these alternative acceptors are more efficient in gasoline degradation, and in fact would not be expected to be, based on microbial metabolism fundamentals. Research into their ability as supplemental electron acceptors is promising, however.

Natural bioremediation of gasoline contaminants does occur, and the design of an effective bioremediation system should be capable of eliminating problems associated with contaminant adsorption, capillary storage, and vapor storage. There are limitations to this technology, including the need for minimum aquifer permeabilities. Additionally, much needs to be learned about contaminant degradation pathways and methods for accelerating the process utilizing alternative electron acceptors. Notwithstanding all of this, the use of biological methods to clean up gasoline contaminated aquifers, and potentially many other organic contaminants, is the most promising technology available today.

REFERENCES

Abdul, S.A., "Limitations of Monitoring Wells for the Detection and Quantification of Petroleum Products in Soils and Aquifers." Ground Water Monitoring Review, 9:4, 90-99 (1989).

Abdul, S.A., "Migration of Petroleum Products Through Sandy Hydrogeologic Systems." Groundwater Monitoring Review, 73-81 (1989).

American Petroleum Institute "Beneficial Stimulation of Bacterial Activity in Ground Waters Containing Petroleum Products." Report No. 4427 API, Washington, D.C. (1975).

American Petroleum Institute "Enhancing the Microbial Degradation of Underground Gasoline by Increasing Available Oxygen." Report No. 4428, API, Washington, D.C. (1982).

American Petroleum Institute "Feasibility studies on the Use of Hydrogen Peroxide to Enhance Microbial Degradation of Gasoline." Report No. 4389, API, Washington, D.C. (1985).

Atlas, R.M., Environmental Protection Agency Report, "Biodegradation of Hydrocarbons in Soil and Groundwater." Hazardous Waste Engineering Research Laboratory, Edison, N.J., PEI Associates, Incorporated, Washington, D.C. (1988).

Barker, J.F., and Patrick, G.C., "Natural Attenuation of Aromatic Hydrocarbons in a Shallow Sand Aquifer." Proceedings of the NWWA/API Conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater: Prevention, Detection, and Restoration, Houston, Texas (1985).

Bartha, R., "Biotechnology of Petroleum Pollutant Biodegradation." Microbial Ecology, 12, 155-172 (1986).

Benefield, L.D., and Randall, C.W., Biological Process Design for Wastewater Treatment, Prentice-Hall, Incorporated, Englewood, Cliffs, N.J., 1-45 (1980).

Bitton, G. and Gerba, C.P., Groundwater Pollution Microbiology, John Wiley and Sons, New York, NY, 1-23, (1984).

Brenoel, M., and Brown, R.A., "Remediation of a Leaking Underground Storage Tank with Enhanced Bioremediation." Proceedings of the 5th National Symposium on Aquifer Restoration and Groundwater Monitoring, National Water Well Association, Worthington, Ohio (1985).

Britton, L.N., Texas Research Institute, Incorporated Report, "Aerobic Denitrification as an Innovative Method For In-Situ Biological Remediation of Contaminated Subsurface Site." Report No. AD-A208-812, Texas Research Institute, Austin, Texas (1989).

Bouwer, E.J., and McCarty, P.C., "Modeling of Trace Organics Biotransformation in the Subsurface." Groundwater, 22:4 (1984).

Conner, R.J. "Case Study for Soil Venting." Pollution ngineering, 20:7 74-78 (1988).

Dragun, J., The Soil Chemistry of Hazardous Materials, Control Research Institute, Silver Spring, Maryland, 221-290 (1988).

Environmental Protection Agency, "Cleanup of Releases from Petroleum USTs; Selected Technologies." Report No. EPA - 530/UST/001, US EPA, Washington, D.C., (1988).

Environmental Protection Agency, "DRASTIC: A Standardized System for Evaluating Groundwater Pollution Potential Using Hydrogeologic Settings." Report No. EPA-500/2-87/035, US EPA, Ada, Oklahoma, (1987).

Environmental Protection Agency, "Protection of Public Water Supplies from Groundwater Contamination." Report No. EPA - 625/4-85/016, US EPA, Cincinnati, Ohio (1985).

Environmental Protection Agency, "Underground Storage Tank Corrective Action Technologies."
Report No. EPA - 625/6-87-015, US EPA, Cincinnati, Ohio (1987).

Environmental Protection Agency, "Leaking Underground Storage Tanks: Remediation with Emphasis on In-Situ Biorestoration." Report No. EPA - 500/2-87/008, US EPA, Ada, Oklahoma (1987).

Environmental Protection Agency, "Processes Affecting Subsurface Transport of Leaking Underground Tank Fluids." EPA Report, US EPA, Las Vegas, Nevada (1987).

Environmental Protection Agency "Water Related Environmental Fate of 129 Priority Pollutants." Report No. EPA-440/4-79-029a, US EPA, Washington, D.C. (1979).

Fogel, S. and Findlay, M. "Bioremediation Techniques for In-Situ Remediation of Soil and Water." Proceedings of the Northeast Regional UST and Hydrocarbon Contamination Cleanup Conference, University of Massachusetts, Amherst, Massachusetts (1988).

Fogel, S., and Findlay, M. "Bioremediation Techniques for In-Situ Remediation of Soil and Water." Proceedings of the Northeast Regional UST and Hydrocarbon Contamination Cleanup Conference, Boston, Massachusetts (1988).

Freeze, R.A. and Cherry, J.A., Groundwater, Prentice Hall, Englewood Cliffs, N.J. 15-75, 314-358, 424-433 (1979).

Ghiorse, W.C., and Balkwill, D.C., "Enumeration and Morphological Characteristics of Bacteria Indigenous to Subsurface Environments." Developments in Industrial Microbiology, 24, 213-223, (1983).

Grbic-Galic, D., and Vogel, T.M., "Transformation of Toluene and Benzene by Mixed Methanogenic Cultures." Applied and Environmental Microbiology 53:2, 254-259 (1987).

Hayes, D. "A Practical Approach to Shallow Petroleum Hydrocarbon Recovery." Groundwater Monitoring Review, 180-184 (1989).

Jamison, V.W., Raymond, R.L., and Hudson, J.O., "Biodegradation of High Octane Gasoline in Groundwater." Developments in Industrial Microbiology, 16, 305-311 (1975).

Keely, J.F., Piwoni, M.D., and Wilson, J.T., "Evolving Concepts of Subsurface Contaminant Transport." Journal Water Pollution Control Federation, 58:5, 349-357 (19).

Keely, J.F., Environmental Protection Agency Report, "Performance Evaluations of Pump and Treat Remediation." Report No. EPA - 540/4-89/005, US EPA, Ada, Oklahoma (1989).

Kostecki, P.T. and Calabrese, E.J., Petroleum Contaminated Soils, Remediation Techniques, Environmental Fate, Risk Assessment, Lewis Publishers, Chelsea, Michigan, 53-81, 211-225 (1989).

Lee, D.C., "Proposed Feasibility Study for an In Situ Bioremediation of a Minnesota Site." Proposal Report, Dupont Environmental Remediation Services, Aston, Pennsylvania (1990).

Lee, M.D. "Biorestoration of Aquifers Contaminated with Organic Compounds." National Center for Groundwater Research-CRC Critical Reviews in Environmental Control 18:1, 29-46 (1988).

Litchfield, C.D., "Present and Future Directions in In Situ Bioreclamation." Proceedings of Hazmat Control, Annual Meeting of the Hazardous Materials Research Council, 1989, 587-596 (1989).

Lyman, W.J., Environmental Protection Agency Report, "Environmental Partitioning of Gasoline in Soil and Groundwater Compartments." Hazardous Waste Engineering Research Laboratory, Edison, N.J., PEI Associates, Inc., Washington, D.C. (1988).

McCarty, P.L., Rittman, B.E., and Bouwer, E.J., "Microbiological Processes Affecting Chemical Transformation in Groundwater.", Groundwater Pollution Microbiology, John Wiley and Sons, Incorporated, New York, N.Y., 90-112 (1984).

McKee, J.E. "Gasoline in Groundwater". Journal of the Water Pollution Control Federation, 44:2, 293-302 (1972).

Nash, J.H., Environmental Protection Agency Report, "Motor Fuel and Organic Chemicals Released in an Underground Environment." Hazardous Waste Engineering Research Laboratory, Edison, N.J., PEI Associates, Inc., Washington, D.C. (1988).

Novak, J.T., Hickman, G.T., Morris, M., Benoit, R.E., "An Investigation of the Biodegradation Potential of Groundwater Contaminants." Virginia Water Resources Research Center, Bulletin 165,
Virginia Polytechnic Institute and State University, Blacksburg, Virginia (1989).

Preslo, L.M. Remedial Technologies for Leaking Underground Storage Tanks, Lewis Publishers Chelsea, Michigan, 37-51. (1988).

Rahn, P.H., Engineering Geology an Environmental Approach, Elsvier Science Publishing Company, Incorporated, New York, N.Y., 189-218 (1986).

Raymond, R.L. "Assessment of Potential for In-Situ Bioremediation." Proceedings of the Joint Meeting of the Society of Industrial Microbiology and DECHEMA, Frankfurt, Germany (1989).

Raymond, R.L., "Bioremediation in the Refinery Environment, a Unique Opportunity to Reduce Cost of Compliance." Proceedings of the National Petroleum Refiners Association Meeting, San Antonio, Texas, (1990).

Reible, D.D., Environmental Protection Agency Report, "Applications of Multiphase Transport Fundamentals to Subsurface Gasoline Movement." Hazardous Waste Engineering Research Laboratory, Edison, N.J., PEI Associates, Inc., Washington, D.C. (1988).

Schultz, B., "Studies on the Bacterial Flora Associated with Subsurface Gasoline Contamination Correlated with Various Site Parameters." Proceedings of the American Society of Microbiologists Meeting, Anaheim, California (1990).

Shorthouse, B., "The Process and Potential of Bioremediation." Remediation, 1:1, 31-40 (1990).

Stover, E.L., "Coproduced Groundwater Treatment and Disposal Options During Hydrocarbon Recovery Operation." Groundwater Monitoring Review, (1989).

Testa, S.M., and Paczkowski, "Volume Determination and Recoverability of Free Hydrocarbon."

Groundwater Monitoring Review, 120-128 (1989).

Westray, S. and Brubaker, G.R., "Design Considerations for In-Situ Bioremediation." Proceedings of the Northeast Regional UST and Hydrocarbon Contamination Cleanup Conference, University of Massachusetts, Amherst, Massachusetts (1988).

Westray, M.S., and Brubaker, G.R., "Design Considerations for In-Situ Bioreclamation." Proceedings of the Northeast Regional UST and Hydrocarbon Contamination Cleanup Conference, Boston, Massachusetts (1988).

Wilson, B.H., Smith, E.B., and Rees, J.F., "Biotransformation of Selected Alkybenzenes and Halogenated Aliphatic Hydrocarbons in Methogenic Aquifer Material: A Microcosm Study." Environmental Sciences and Technology, 20, 997-1002 (1986).

Wilson, J.L., Environmental Protection Agency Report, "Transport of Organics." Hazardous Waste Engineering Research Laboratory, Edison, N.J., PEI Associates, Inc., Washington, D.C. (1988).

Wilson, J.T., "In-Situ Biorestoration as a Groundwater Remediation Technique." Groundwater Monitoring Review, (Fall, 1986).

Wilson, J.T., "Enumeration and Characterization of Bacteria Indigenous to a Shallow Water Table Aquifer." Groundwater, 21:2, (1983).

Wilson, J.T., McNabb, J.F., Wilson, B.H., and Noonam, M.J., "Biotransformation of Selected Organic Pollutants in Groundwater." Developments in Industrial Microbiology, 24, 225-233, (1983).

Yaniga, P.M., and Mulry, J., "Accelerated Aquifer Restoration: In-Situ Applied Techniques for Enhanced Free Product Recovery/Adsorbed Hydrocarbon Reduction via Bioreclamation." Proceedings of the NWWA/API Conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater: Prevention, Detection, and Restoration, Houston, Texas (1984).

Yanigita, T., Natural Microbial Communities, Ecological and Physiological Features, Japanese Scientific Societies Press, Tokyo, Japan, 171-213 (1991).