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Final Report
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The contents of this report reflect the views of the authors, who are responsible for the facts and the accuracy of the data and information presented herein. The contents do not necessarily reflect the official views or policies of the DOTD, FHWA, and Louisiana Transportation Research Center. This report does not constitute a standard, specification or regulation.

APRIL, 1994
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ABSTRACT

The extent and duration of pollution from herbicide spills and deliberate applications is related to properties of the herbicide and soil. Due to potential negative impacts on traffic flow and public safety following roadside spills, expedient cleanups within hours are preferred to cleanups requiring days or weeks. Isolated microorganisms, adapted to specific organic compounds, have the potential to quickly remediate contaminated soils. Biogeochemistry and physical processes of the roadside environment control the rate of accelerated biodegradation. Factors determining the success of accelerated biodegradation include the complex interactions between: herbicide and soil (Chapter 4), bacteria and soil (Chapter 5), and bacteria and herbicide (Chapter 6). Objectives of this study included the development of experimental procedures and mathematical models to determine the sorption, transport, and biodegradation rates of herbicides in soil using adapted microorganisms. Soil column and flume experiments simulated one dimensional and two dimensional transport of bacteria and 2,4-D, a widely used roadside herbicide. The adapted microorganisms were an isolated strain of Pseudomonas capable of degrading 2,4-D in aqueous solution. 2,4-D, a hydrophilic herbicide, was separated with liquid chromatography and measured with ultraviolet absorbance at 230 nm. A typical silty loam soil was selected to represent roadside conditions in Louisiana. Soil column models show the influence of preferential flow through the largest continuous pores which absorb less 2,4-D than batch studies predict. The hydraulic properties of the soil dominate the transport of the hydrophilic herbicide in one- and two-dimensional experiments. Heterogeneities in the soil create stagnant zones where herbicide transport is limited to diffusion. In batch studies, the addition of surfactant decreased significantly the number of bacteria which adsorb to the soil. High bacterial concentrations (10^9 cfu/ml) travel greater distances into soil columns than low concentrations (10^7 cfu/ml). Continuous injection of bacteria into soil is not beneficial compared to intermittent injection and causes plugging which decreases bacterial concentrations at greater depths. A mathematical model for the deposition of bacteria in saturated or unsaturated soil predicts transport rates of bacteria in one dimension. A numerical solution using the orthogonal collocation method is better than
a method using Galerkin finite element for an analytical problem of unsaturated flow. Four parameters: dispersion coefficient, clogging coefficient, declouging coefficient, and velocity predict the rate of spatial penetration of bacteria into soil. Batch biodegradation studies show that the adapted bacteria are able to entirely degrade up to 500 mg/L of 2,4-D within 24 hours. Biodegradation in one and two dimensions is only effective in the most permeable regions. Preferential flow of water in large soil pores determines the effectiveness of using acclimated microorganisms. Accelerated bioremediation using bacterial seeds is economically feasible for small spills affecting the topsoil and requiring quick remediation. Specialty bacteria should also be used to clean up organic spills when the natural soil microorganisms have been destroyed by the spilled herbicide. A soil slurry treatment system is recommended in order to uniformly distribute oxygen and bacteria to all of the contaminated soil. For deeper spills, groundwater should be pumped and treated with acclimated bacteria in an activated sludge process. Costs of bioremediation are generally equal or less than alternative treatment methods such as landfiling or incineration. Specialty bacteria are well suited for spills which incur high daily costs of treatment, as is the case when the spill interferes with traffic or a public water supply. Three parameters permit managers to compare the costs of natural and seeded bioremediation. The essential economic factors are: 1) the difference in treatment time between natural biodegradation and seeded biodegradation, $\Delta t$, 2) the cost of supplying the seed, $C_s$, and 3) the daily costs of treatment, $C_D$. If the daily costs, $C_D$, of treatment are greater than $C_s/\Delta t$, then using acclimated bacteria is less costly than using natural bacteria. Field monitoring of the impact of roadside chemicals should be conducted on the most vulnerable regions (areas with the highest value of the pollution index) in Louisiana.
IMPLEMENTATION STATEMENT

The results of this study can be used to clarify the relevant technical problems encountered in the treatment of roadside spills. Model parameters, which predict the ability to supply acclimated bacteria to contaminated soil, should be linked to roadside soil types in order to select and apply effective remedial actions. The experimental methods developed may be used to study accelerated bioremediation of other herbicides. Future actions should include the a field demonstration of applying acclimated bacteria to clean up both large and small spills of herbicides in topsoil and subsoil environments. The success of batch biodegradation experiments indicates that the addition of acclimated bacteria to soil slurries made from excavated soil will rapidly clean small spills which only penetrate the topsoil. Initially, a simple field demonstration using acclimated bacteria in a soil slurry to treat an excavated topsoil contaminated from a small herbicide spill should be analyzed for effectiveness, feasibility, and required duration. The demonstration scale project will enable the input of transportation workers to be obtained so that the most appropriate means of implementing an accelerated biodegradation program for herbicides can be developed. A knowledge-based system, based on linking the technical information of this report with the practical experiences of transportation workers, which considers the spilled chemicals present at a particular roadside location, would expedite selection of appropriate treatment methods.

Field evaluation of surfactant treatment to enhance penetration of bacteria into deeper spills in heterogeneous soils should be carried out. Field studies of accelerated biodegradation of large spills in heterogeneous soils should not be conducted until methods are developed to enhance treatment in stagnant regions with low permeability. Large spills which enter the B horizon may not be suitable for accelerated biodegradation due to the difficulties in supplying beneficial bacteria and oxygen to stagnant regions. Optimal methods of field injection of beneficial bacteria need to be developed.

The unique hydrology of Louisiana, characterized by high water tables and the dominance of wetlands, affects the fate of herbicides. Field monitoring of the impact of roadside chemicals on the most vulnerable regions of Louisiana should be conducted. These areas can be identified using the pollution index presented in Chapter 7. Areas
with the highest value of the pollution index are those most in need of careful application and monitoring. With limited financial resources, a worst case monitoring effort is very economical and practical. The index also helps to select the proper herbicide for a given roadside in order to minimize environmental risks.

In order to compare the costs of natural and seeded bioremediation, three parameters should be quantified. The essential economic factors are: 1) the difference in treatment time between natural biodegradation and seeded biodegradation, Δt, 2) the cost of supplying the seed, C_S, and 3) the daily costs of treatment, C_B. If the daily costs, C_D, of treatment are greater than C_S/Δt, then using acclimated bacteria is less costly than using natural bacteria.
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1. INTRODUCTION

1.1. Problem Statement and Limitations

Herbicides are being used with increasing frequency in the management of roadside vegetation. In addition, unintended spills add to the concern about herbicides in the roadside environment. Herbicides may be used alone or in combination with others. Large amounts of money are spent on herbicides such as: (a) 2,4-D, (b) MSMA, (c) OUST, (d) GARLON 3A, (e) RODEO, (f) ROUNDUP, and (g) CAMPAIGN.

Herbicides may cause adverse environmental impacts at the point of application on plants, animals, and humans. The adverse impact may be felt elsewhere also as the herbicide and its breakdown products can be transported by surface water, soil water, and groundwater.

The advances in applied microbiology and environmental engineering made it possible to use microorganisms to accelerate the degradation of organic contaminants in artificial laboratory environments. The microorganisms metabolize the organic pollutant, in our case the herbicide studied. In order to minimize the potential hazard of herbicides in the roadside environment, it is necessary to develop simple and easy methods to utilize microorganisms for degrading herbicides when they are present in excessive concentrations. Hence, experimental procedures need to be developed which resemble natural environments as closely as possible. Once such procedures are established, field level experiments can be conducted to solve real world problems.

In the state of Louisiana herbicides are used to provide control over excessive growth of brush and plants in the roadside environment. A mixture of the herbicides mentioned above are applied depending on the location and time of the year. This implies that any management practice involving microorganisms has to be adapted to the specific conditions of the spraying areas and time. Due to the complexity of mass transfer phenomena in natural environments, this research is limited to the existing knowledge about the transport and sorption mechanisms of both herbicides and bacteria.
The mechanisms controlling biodegradation of xenobiotic chemicals in subsurface environments are still subject to intensive basic research (1). Hence, the conceptualization and design of experiments aimed at the development of practical applications in bioremediation are constrained by the current knowledge; the knowledge of (i) herbicide sorption, degradation, and transport and (ii) the mobility and ecology of the microorganisms applied to enhance biodegradation. In addition, bioremediation measures cannot be designed without understanding the behavior of herbicides in soils. Since environmental cleanups are urgently needed, it is important to find suitable empirical methods which can be quickly developed to assess the potential bioremediation techniques. In this context, the results of present research are limited to testing existing microorganisms for their suitability to degrade 2,4-D in soil columns and a 2-dimensional soil flume. Presently, attempts are made to create hypothetical scenarios in the laboratory which are relevant in real world soils.

1.2. Review
1.2.1. The Roadside Environment

The roadside environment has a unique character, that is, the roadside can be viewed as the interface between the road and the adjacent environment (agricultural land, forest, residential neighborhoods and others). In addition to the release of potentially hazardous substances, such as heavy metals (Pb, Cd, Ni), nitrous oxides, carbon monoxides, and other emissions by vehicles, applications of herbicides to the roadside may contribute significantly to soil and groundwater contamination. Herbicides may be transported through the atmosphere as fine aerosols or as part of aquatic solutions through surface and subsurface runoff. In both cases the roadside is the intermediate receptor of such substances.

In order to ensure that the roads are long lasting and safe for traffic, roads are designed to handle intensive rainfalls, that is, to provide sufficient surface runoff. Therefore, the roadside is usually a sloped surface with a ditch to ensure fast runoff. On
one hand, the roadside functions as a runoff channel. On the other hand, the pollution hazard should be minimized. Hence, the roadside should also be a pollution control buffer. The former aspect of roadside requires the knowledge of the chemical and physical properties of the soil used. In general, roadside soils are either disturbed or artificially textured soils. The soil should have a large infiltration capacity in order to avoid erosion caused by surface runoff. In addition, the soil should support plant growth, which supports the stability and resistance towards erosion. The clay fraction can be expected to make up a small portion of the soil and the mineralogical composition should exclude 2:1 expanding clay minerals. In order to support plant growth, the soil should have nutrient storage capacity which can be given by soil organic matter (OM). The OM content will increase with increasing biomass production and time.

1.2.1.1. Weed Management and Hydrologic Cycle

In addition to agricultural purpose, the use of herbicides has application in maintaining the right-of-way (2). Vegetation control on the right of way is important for both aesthetic and economic reasons (2). As a result, vegetation management on the roadsides is becoming an integrated division of land management. Herbicides are being extensively used in many states in the United States for vegetation management on the roadside. Table 1 shows the different types of herbicides and their application rates used on the roadside in the state of Louisiana. According to estimates reported in the 1962 Northeast Weed Control Conference, about 33 million acres of land were treated for vegetation management in connection with maintenance of highways and railroads nationwide. In 1965 about 15 million acres of land were utilized for roadside and highway combined and the figures have grown dramatically in recent years (2). More recently, a survey of the roadside maintenance programs conducted by the Transportation Research Board in 1987 updated the information on herbicides. In particular, the total area and mileage of managed roadside as well as the number and types of herbicides were reviewed. With 17,080 miles of managed roadside, Louisiana lies about 23% above the national average. The total managed area is 94,879 acres, which is about 26% below the national average. Approximately 52% of this area is managed by using
# TABLE 1
DIFFERENT HERBICIDES, THEIR APPLICATION RATES AND PERIOD OF APPLICATION
USED FOR VEGETATION MANAGEMENT ON THE ROADSIDE
IN THE STATE OF LOUISIANA

<table>
<thead>
<tr>
<th>Herbicide name</th>
<th>Rate per acre</th>
<th>Est. cost per acre</th>
<th>500 Gal. tank mix</th>
<th>Nov-Dec</th>
<th>Jan-Feb</th>
<th>Mar-Apr</th>
<th>May-Oct</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oust</td>
<td>1 oz.</td>
<td>$7.50</td>
<td>16 oz.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>2 oz. of Oust to be used where Oust is required in Nov and Dec. In areas where there is heavy conc. of Johnson grass, ½ oz. per acre must be used. 1 oz. must be used after Johnson grass has been eliminated.</td>
</tr>
<tr>
<td></td>
<td>1½ oz.</td>
<td>3.75</td>
<td>24 oz.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 oz.</td>
<td>15.00</td>
<td>32 oz.</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-D</td>
<td>2 qt.</td>
<td>$3.00</td>
<td>8 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Combination of 2,4-D and Roundup must be used only in southern part of state and when temp reaches 75°C and above.</td>
</tr>
<tr>
<td>Roundup</td>
<td>1 pint.</td>
<td>6.07</td>
<td>2 gal.</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlon3A</td>
<td>1 qt.</td>
<td>$11.00</td>
<td>4 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Only to be used in northern part of the state where 2,4-D is restricted and when temperature is &gt; 75° C.</td>
</tr>
<tr>
<td>Roundup</td>
<td>1 pint.</td>
<td>6.07</td>
<td>2 gal.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.S.M.A</td>
<td>2 qt.</td>
<td>$5.77</td>
<td>8 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Only to be used in southern part of the state where 2,4-D is not restricted, and on very rare occasions where Roundup will not do as good a job.</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2 qt.</td>
<td>3.00</td>
<td>8 gal.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.S.M.A</td>
<td>2 qt.</td>
<td>$5.77</td>
<td>8 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Only to be used in the northern part of state where 2,4-D is restricted.</td>
</tr>
<tr>
<td>Garlon3A</td>
<td>1 qt.</td>
<td>11.00</td>
<td>4 gal.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campaign</td>
<td>48 oz.</td>
<td>$7.50</td>
<td>6 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Can be used in southern section of state in lieu of 2,4-D and Roundup mixture. Heavy concentration of vines and woody plants need 1 pint of 2,4-D.</td>
</tr>
<tr>
<td>Rodeo</td>
<td>1 pint.</td>
<td>$11.50</td>
<td>2 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>To be used on the slopes and in water where Bermuda grass should not be destroyed. To be used under bridges and in water for control of vegetation.</td>
</tr>
<tr>
<td></td>
<td>1 qt.</td>
<td>23.00</td>
<td>4 gal.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roundup</td>
<td>2 qt.</td>
<td>$47.00</td>
<td>8 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>To be used on shoulders and when temp &gt; 70°C.</td>
</tr>
<tr>
<td>HyvarXL</td>
<td>10 gal.</td>
<td>$276.00</td>
<td>120 gal.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Used for total sterilization of soil as in storage yards.</td>
</tr>
</tbody>
</table>
selected herbicides. With 17% of the total budget for roadside maintenance, the herbicide management cost is higher than the national average of 13.8 percent. Also, it is noteworthy that the application of herbicides in Louisiana has increased 230% over the last 10 years while the mowing activity has decreased 46%. Obviously, the increase of herbicide use can be attributed to a shift from mowing to herbicide management practice. In particular, the herbicides are used to eliminate unwanted species such as Johnson, Dallas, and Vasey grasses and to reduce mowing frequency. Also, the application of 2,4-D, as part of mixed herbicide solution, has been reported with good success.

The use of herbicides on the roadside has been discussed by Iurka (3) and Zukel and Eddy (4) for the following reasons: 1) To increase the highway safety by reducing driving hazards by allowing unobstructed view of warning signs, traffic lights and the views of curves; 2) To control weeds on the roadside for the purpose of reducing the number of mowings; 3) To keep the water drainage areas free of weeds and brush which impede water flow. Iurka (3) has also discussed the hazards concerned with herbicide application along roadside due to drift, volatility, wash-off, leaching and public reaction to high degrees of brown-out due to the killing of the vegetation. The fate of the herbicides which are sprayed on the roadside for vegetation management has been schematically illustrated in Figure 1.

1.2.1.2. Soil Properties

The soil represents an interface between the atmosphere and subsurface water. The physical characteristics of the soil will determine the transport behavior of contaminants to the groundwater. The following physical parameters influence contaminant transport:

- hydraulic properties (hydraulic conductivity and water retention curve)
- dispersivity
- bulk density
- clay content and mineralogy
- organic matter content
- texture
- structure
Figure 1. Schematic drawing of water and herbicide fate in a roadside environment.
For modeling purposes one needs to know the hydraulic properties, such as the hydraulic conductivity and water retention curve. Furthermore, the mixing regime of the solutes is important to make any prediction. In particular, the structure and texture will control to a large extent whether or not the herbicides will move as a homogeneous concentration front through the soil. Field experiments have, however, revealed that bypass or preferential flow contributes significantly to the solute distribution in the soil. In fact, concentration profiles may exhibit up to two distinct concentration peaks, each propagating at a different solute flux. These phenomena complicate the development of predictive models since no a priori knowledge of bypass flow is available (5). More details of the transport behavior of solutes are presented in section 1.2.2.3.

In contrast to the purely physical properties controlling the convective and diffusive processes, soils can be viewed as constantly operating chemical reactors. In order to understand the chemical reactions taking place in a soil, one can classify the soil components into organic and inorganic materials. First, the inorganic substances are largely made of silicates, with quartz as one of the most common components. Other important solids are phyllosilicates such as 2:1, 1:1, and 2:1:1 clay minerals, including montmorillonite, kaolinite, and chlorite, respectively (6). The smectites are negatively charged and, therefore, adsorb cations. The negative charge is due to isomorphous substitution of Mg and Al within the octahedral and tetrahedral sheets, respectively. The charge from those minerals are commonly referred to as permanent charge. In contrast, oxides and hydroxides of Fe and Al possess very little permanent charge. Instead, their charge is due to surface functional groups involving water. These minerals produce variable charge soils, since the charge is dependent on the pH. Organic matter behaves similarly to oxides and hydroxides with respect to their charge characteristics. The charge is dependent on the type and amount of surface functional groups present in the soil. The most important key indicators for the chemical behavior of soils are as follows:

- Mineralogy
- pH
- Cation exchange capacity (CEC)
- Redox potential.
The different physical and chemical properties of soils are not homogeneously distributed in the soil profile. In fact, soil is identified and classified by horizontal layer which is located parallel to the soil surface. They differ in properties and characteristics from the adjacent layers above and below and are commonly referred to as soil horizons. Four different master horizons important to our study of soil are distinguished. They are designated by the letters A, E, B, and C (6). These appear in this order from the top to the bottom in a soil profile. Sub layers or distinctions within these master horizons are designated by the lowercase letters. A typical Loring silt loam has A, E, B, and C horizons of soils in the order from the top. The Loring silt loam soil profile is illustrated in Figure 2.

The A horizons are the top most mineral horizons. They contain a strong mixture of partially decomposed (humified) organic matter which tends to impart a darker color than that of lower horizons. Soil of A horizon is commonly referred to as 'topsoil'. E horizon soil is characterized by the maximum illuviation of the silicate clays iron and aluminum oxides, and this layer commonly occurs above the B horizon and below the A horizon.

The subsurface B horizon soil includes layers in which illuviation of materials has taken place from both above and below. Illuviation is the process in which materials are carried from an overlaying layer, are precipitated from solution, and are deposited in the underlying layer. In the B horizon we have the maximum accumulation of the materials such as iron, aluminum oxides, silicate clays, calcium carbonates, calcium sulfates and other salts. The B horizon is sometimes referred to as "subsoil". C horizon is a mineral horizon and is relatively unaffected by the biological activity and is lacking in properties diagnostic of an A or B horizon. The lowercase letter p indicates plowing or other disturbance and the lowercase letter t indicates the accumulation of silicate clays.

1.2.2. Herbicide Behavior
1.2.2.1. Sorption

Retention is one of the important factors which determines the fate of herbicides in the soil environment. Retention is the ability of the soil to hold a pesticide or other
Figure 2. Soil profile showing different horizons of a Loring silt loam soil.
organic molecule. Retention primarily refers to the adsorption process. The retention process strongly influences the chemical transport of the pesticide to surface water, groundwater and atmosphere.

Different intramolecular forces that attract molecules to the interface and retain them on the surface have been termed as mechanisms of adsorption. Organic compounds can be sorbed by physical/chemical bonding such as van der Waal forces, H-bonding, dipole-dipole interactions, ion exchange, co-valent bonding, ligand exchange, cation bridging and water bridging with varying degrees of strengths of interactions (7). For any given compound, there are several mechanisms responsible for sorption to the soil. Organic matter may be sorbed initially by sites that provide the strongest mechanism, and then by weaker mechanisms. The different possible mechanisms are:

- London van der Waals bonds: The London van der Waals forces are short range bonds that result from the dispersion forces.

- Hydrogen bonds: Hydrogen bonds are produced by the electrostatic attraction between an electro-positive hydrogen nucleus and exposed electron pairs on electro negative atoms. These bonds are stronger dipole-dipole interactions than van der Waal interactions. Hydrogen bonds can occur both intra- and inter-molecularly.

- Ion-exchange: There are two types of ion-exchange mechanisms, the anion exchange and cation exchange. Anion exchange involves the exchange of an anion at the binding site, due to nonspecific attraction of an anion to a positively charged site on the surface of the soil. These types of mechanisms involve a much stronger bonding. The cation exchange process is more prevalent because of the presence of a large number of negatively charged sites associated with clays and organic matter. This type of mechanism is uncommon among pesticides, since there are few positively charged pesticides.

- Ligand exchange: The Ligand exchange is a stronger mechanism than the anion exchange mechanism. During this process, a complex is formed by the exchange of a functional group on the surface of a soil mineral. This
mechanism has been proposed for weak acids and anions on hydrous oxide surfaces (8).

Acidic pesticides, such as 2,4-D and 2,4,5-T, can ionize in aqueous solutions forming anionic species (9). The mechanisms of sorption for these compounds are proton association and van der Waals sorption for the molecular form (9). Hydrogen bonding and electrostatic interactions are other possible mechanisms for sorption.

1.2.2.2. Degradation

Once the pesticides are introduced to the soil environment, they are likely to undergo various transformations. One of the mechanisms of the breakdown is by photochemical degradation. Phenoxy-alkaline acids are capable of absorbing the radiation found in the sunlight and undergo photochemical decomposition. Their breakdown in irradiated aqueous solutions has been studied (10). However, the photochemical breakdown of phenoxy-alkaline acids by sunlight at the soil surface is not considered to be a major source of herbicidal loss (10).

Another important process responsible for the breakdown of these compounds is microbial degradation. Among the different biological processes, microbial metabolism is the primary force in the pesticide transformation or degradation (11). Microorganisms are very important catalysts in degrading a vast array of organic pesticide molecules in the terrestrial and aquatic ecosystems (11). There are five processes involved in the microbial transformation of the pesticides:

- **Biodegradation**: In this case the pesticide molecule acts as the energy source of the substrate for the growth of the microorganisms.
- **Cometabolism**: Transformation is by metabolic reactions, but the pesticide does not act as the energy source for the growth of the microorganisms.
- **Polymerization**: Different molecules of the pesticides are linked together and the pesticide molecules are linked to the naturally occurring compounds.
- **Accumulation**: In this case the pesticide molecule as a whole is incorporated in the body of the microorganism.
Secondary effects of the microbial activity: Here the transformation is due to change of pH, redox conditions, reactive products and other processes brought about by the microbial activity.

Microbial transformation may involve more than one of the above processes and can yield various products from the same initial compound under various environmental conditions, since the environmental parameters change with different conditions. The transformation processes can be mediated by one type of microorganism or can result from the combined effects of several types of microorganisms.

The use of biological processes to degrade waste products is not new. Microorganisms have long been used to detoxify sewage. Microbial systems are used to treat manufacturing wastes to remove parathion (12), phenols and Trifluralin (13). The approach by Audus (14) to removing 2,4-D from soil has been proven successful by a number of other researchers. The disappearance of DDT from soil was proved by Kearney et al. (15) by injecting DDT-degrading Aerobacter aerogenes. Clark and Wright (16) demonstrated the detoxification of isopropyl-N-phenyl-carbamate propan (IPC) in soil by inoculating IPC-utilizing Arthrobacter and Acromabacter species. The acceleration of the hydrolysis of parathion was observed by Sethunathan and Rajaram (17) by inoculating a bacterial culture capable of hydrolyzing parathion. When adding a combined culture of Pseudomonas stutzeri and P. aeruginosa to soils contaminated with parathion, there was 85% mineralization of parathion in 4 days (18). The degradation of pentachlorophenol (PCP) in contaminated soil increased tenfold with the inoculation of PCP degrading Arthrobacter (19). The microbial detoxification of 2,4,5-T was demonstrated by Chatterjee et al. (20), using a strain of P. cepacia which removed about 95% of 2,4,5-T within one week from the soil treated with the chemical.

An alternative technique for microbial detoxification of the pollutants has been suggested by Kunc et al. (21) in which stimulation of the mineralization of the pollutant in the soil is enriched with saccharides and other organic substrates. Kunc et al. (21) reported the that mineralization of 2,4-D was enriched with saccharides and other organic substrates.
There are limitations to the use of microorganisms to degrade toxic chemicals. The microbial detoxification involves whole cells which will be subjected to chemical shock, extremes of pH, and metabolic inhibitions. In many cases these factors may destroy or harm the microorganisms and prevent their degradation capabilities. Therefore, for successful detoxification of the pesticides and other pollutants, not only must proper microorganisms be selected, but conducive environmental conditions must be maintained. According to Goldstein et al. (22), there are several reasons why microorganisms do not metabolize the pollutants in the environment:

- the concentration of a xenobiotic may be too low to support the growth of the microorganisms
- the microorganisms may be susceptible to predators or toxins
- other organic matter may be used by the microorganisms in preference to the pollutants
- movement of the microorganisms may be difficult in the soil media.

The importance of soil microorganisms for the degradation of 2,4-D and other herbicides was demonstrated with soil perfusion experiments (23). Soil columns were continuously percolated with aqueous solutions containing high concentrations of the herbicide and the effluent monitored for the remaining herbicide (24). There were slight reductions in the concentration at the initial stages due to adsorption, followed by a lag phase of two weeks for 2,4-D degradation by the soil microorganisms (23). After the lag phase, further applications of herbicide were rapidly degraded.

Microbes in the natural environment do not have the necessary enzymes to degrade these compounds as the microorganisms were not exposed to such manmade herbicides (25). The lag phase is due to the fact that the natural soil microorganisms are incapable initially of degrading the herbicides. Due to continuous exposure to these compounds, certain groups of microorganisms develop an enzymatic system which is resistant to toxic compounds and can degrade them (25). The lag phase of two weeks is crucial because in the soil environment during the period of these two weeks (for 2,4-D), the herbicides can be transported by different processes like runoff and percolation, which will lead to contamination of groundwater and surface water bodies as mentioned.
earlier. Thereby, emphasis should be put on rapid breakdown of these compounds in the soil environment.

Two Pseudomonas isolates capable of degrading 2,4-D have been isolated in the environmental engineering laboratory at Louisiana State University. These are gram-negative, non-fermentive, motile rods (25). These strains of microorganisms will be used to study accelerated degradation of 2,4-D in soil media.

1.2.2.3. Transport

In the conventional solute transport equation, one of the major components is the assumption of local equilibrium. For this to be valid, sorption must be much faster than the other processes, such as advection and hydrodynamic dispersion. Lab and field investigations have shown that this is not valid all the time (26). According to Brusseau and Rao (26), data from sorption experiments have shown that there is a two stage approach to equilibrium, a fast initial uptake followed by and a slower uptake. In recent years, a bicontinuum model consisting of both a physical and chemical approach (depending upon which process is rate-limiting for sorption non-equilibrium) has been introduced (26).

(1) Chemical model: In this model the non-equilibrium is assumed to result from time-dependent sorption. Here it is hypothesized that the sorbent has two types of sorption sites. In the first type of site, the sorption is said to be instantaneous and in the second type of site it is rate-limiting. This model has been developed by Selim et al (27) and Cameron and Klute (28), known as a "Two-Site" model. The Two-Site model has been able to explain the non-equilibrium data better than other models.

(2) Physical model: In these models the sorbent-solvent interface is assumed to be instantaneous; the rate at which the solute is transported to and from this interface controls the sorption rate (26). The water present in the soil is designated by two regions, the mobile region and the stagnant or immobile region. Advective-dispersive solute transport is dominating in the mobile region, whereas in the immobile region, solute transport is controlled by diffusion.
The solute transfer between the mobile and the immobile regions can be explained by three different approaches and are discussed as 1) the physical diffusion model 2) the physical mass transfer model, and 3) the effective dispersion model. The concept of mobile and immobile regions is represented in Figure 3. According to the physical diffusion model, there are several individual mechanisms involved in the solute-transfer process. They are advective-dispersive transport from the bulk solution to the layer of water surrounding the sorbent (known as the boundary layer), diffusive transport across the adsorbed water (known as film diffusion), and pore and/or surface diffusion within the immobile region (also described as intra-aggregate diffusion). In the initial stages, film diffusion seems to control the solute uptake. However, for the majority of the reaction period, intra-aggregate diffusion seems to control the solute uptake. Therefore, the intra-aggregate diffusion is rate limiting during sorption.

Since it is difficult to analyze the pore geometry of the soil, the physical transfer model simplifies the diffusion model by replacing the description of the diffusive transfer by a kinetic mass-transfer expression. In this case, the actual description of the structure of the porous medium is eliminated. A solute transfer is assumed to be a function of the difference of the solute concentration between the mobile and immobile regions. The concentration of the solute in the immobile region is assumed at an average value, which means that this region is a perfectly mixed region. This assumption is in contrast to the physical diffusion model in which a concentration gradient is described by the diffusion model.

In the effective dispersion model, a lumped or an effective dispersion coefficient is used in the convective-dispersive model. This effective dispersion coefficient accounts for the diffusive transfer between mobile and immobile regions.

1.2.2.4. 2,4-Dichlorophenoxyacetic Acid (2,4-D)

The herbicide 2,4-D is chosen in this study because it is one of the most commonly used herbicides for roadside treatment in Louisiana and the United States. In fact, a survey of the roadside maintenance programs conducted by the Transportation Research Board revealed that 2,4-D is second in the list of the 10 most used herbicides.
Figure 3.  Schematic representation of inter- and intra-aggregate porosity of the Two-Region model.
The chlorine-substituted phenoxyacetic acids, such as 2,4-D, were introduced as selective weed killers at the end of World War II, following the publication of secret wartime research on their growth regulating and herbicidal activities (29, 30). The structure and some of the physical properties of 2,4-D are tabulated in Table 2.

The chlorophenoxy herbicides, 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) have been extensively used for several decades as defoliants and growth regulators for selective control of weeds and other vegetation (31). Since large quantities of 2,4-D are applied as a herbicide to soil, its fate and the products of its metabolism have attracted considerable interest (32, 14, 33). When phenoxy herbicides are applied to the soil, 2,4-D persists in the soil for 2 weeks and 2,4,5-T for about two months (34, 33). The rate of breakdown of 2,4-D was found to be proportional to the number of aerobic bacteria. The adapted soil microorganisms could persist for nearly 12 months after being applied to soil that has been contaminated by 2,4-D (35).

Ogram et al. (36) studied the effects of sorption on the biological degradation rates of 2,4-D in soils. It was revealed that the 2,4-D which was adsorbed was completely protected from biological degradation, even though sorbed bacteria were also capable of degrading 2,4-D.

1.2.3. Bacterial Fate in Soil
1.2.3.1. Occurrence of Bacteria in Subsurface Environments

Reports of microbiological activity in the subsurface date back as early as the beginning of this century (37). Since then, the area of subsurface microbial ecology has been evolved and considerable progress has been made. Bacteria occur as colonies attached to solid surfaces in soils and aquifers, which may form so called biofilms or micro colonies (38). Also, bacteria may form consortia of different symbiotic species which may be found in anaerobic environments. The number of bacteria was reported to decrease with depth in soils (< 2 m). However, when bacterial populations were measured in aquifers up to a depth of 264 m, no correlation between depth and population size could be established (39). Rather, a weak correlation between texture
### TABLE 2
THE STRUCTURE AND SOME PHYSICAL PROPERTIES
OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Mol. Wt (g)</th>
<th>Melting Point (°C)</th>
<th>Solubility</th>
<th>pK* (°C)</th>
<th>Acute oral dosage, LD&lt;sub&gt;50&lt;/sub&gt; mg/kg*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>221</td>
<td>140.5 (139-139.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water ppm*</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Organic g/100 ml</td>
<td></td>
<td>CCl&lt;sub&gt;4&lt;/sub&gt;, 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>375</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ether, 27</td>
<td></td>
<td>500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acetone, 85</td>
<td></td>
<td>300-1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.22</td>
</tr>
</tbody>
</table>

* Different values of the same property are reported from different sources.
and population occurred. In particular, coarse textured soil and sediments with larger pore sizes seem to have larger bacteria numbers, whereas clays seem to inhibit bacterial growth (39).

In general, the size of subsurface bacteria ranges from 0.5 to 1.0 microns and occurs as gram-positive, spherical, or rod-shaped cells (40). Yates and Yates (41) listed 51 cases where the horizontal and vertical movements of bacteria were monitored. Out of the 51 studies, 19 monitored vertical movement with a minimum and maximum travel distance of 0.1 and 12 m, respectively. In contrast, the maximum and minimum travel distance of the horizontal studies were 0.5 and 1000 m. Generally, the extent of the horizontal movement seems to exceed that of the vertical movement by an order of magnitude. The migration and survival are influenced by several factors, such as temperature, moisture content, hydraulic conditions, pH, organic matter, salt species and concentration, bacterium type and activity (41).

1.2.3.2. Environmental Factors Controlling Bacteria

As mentioned above, the temperature influences the bacterial survival significantly. Particularly, at low temperatures the bacteria can survive longer. For example, Salmonella typhi survived for 9 weeks at 0 °C, but only 2 weeks at 37 °C (42). Similar findings have been reported for fecal coliform bacteria (43, 44). Also, at and below freezing temperatures the bacteria may exist for years.

While the temperature is a climatic variable, the influence of microbial activity is an ecological aspect of bacterial behavior. By comparing the survival of selected bacteria under sterile and nonsterile conditions, it has been shown that microbial activity decreases the survival time. Antagonisms of competing bacteria have been reported for S. typhi by Proteus vulgaris and Pseudomonas flourescens (45, 46). Also, protozoa may adversely affect the persistence of bacteria (47). The competition for nutrients of different microorganisms may contribute to the observed phenomena (48). The climatic conditions as well as the hydraulic properties control the moisture content in soils. Bacterial survival is strongly dependent on the moisture content and therefore related to the water characteristic curves. For example, Escherichia coli had optimum survival
conditions between 10 and 40% degree saturation \((49)\). The movement of bacteria in unsaturated soils is restricted by the limited amount of mobile water \((50)\). The mobile water is not only controlled by the textural and structural soil properties, but also by the rainfall amount and intensity. Hence, climatic factors and soil hydraulic properties together control the migration caused by convective water transport. The soil texture and structure can also be related to the bacterial mobility. Because bacteria can be viewed as charged particles in the soil matrix, interactions between soil colloids with bacteria may contribute significantly to the screening or filtration and adsorption behavior. In fact, clayey soils adsorb and restrict the movement of bacteria, whereas coarse textured soils permit greater mobility \((41)\). While clay minerals contribute to electrostatic adsorption to bacteria, organic matter controls to a large extent the nutrient availability for bacteria. Besides the chemical and biological importance of organic matter, the physical properties are also influential. In fact, organic matter may cause the formation of bacterial mats which greatly limit the mobility of bacteria.

Chemical factors controlling bacteria survival and mobility are the pH, the salt species, and concentration \((41)\). Generally, acid pH in the range from 3 to 4 will adversely affect the bacterial survival. The optimum pH for enhancing bacterial activity in soils lies between 5 and 8, depending on the species and the environment \((51)\). Also, strongly alkaline conditions may inactivate bacteria. Since the pH controls the nutrient absorption, the adverse effects of low and high pH may be caused by nutrient unavailability. Also, soils contain variable charged surfaces which exhibit positive surface charge at low pH and negative surface charge at high pH. Since bacteria are negatively charged, the bacteria adsorption increases with decreasing pH. In this context, the cations present in the soil influence the colloidal properties of bacteria \((52)\). Especially divalent exchangeable cations, such as calcium and magnesium, may cause bacteria to associate with soil solids more easily. Moreover, high ionic strength solutions of divalent cations may even enhance this phenomenon, whereas low ionic strength may induce higher bacterial mobility \((52)\). Table 3 summarizes the effects of these factors on survival and migration of bacteria.
TABLE 3
FACTORS INFLUENCING THE FATE OF BACTERIA IN SUBSURFACE ENVIRONMENTS (41)

<table>
<thead>
<tr>
<th>FACTORS</th>
<th>INFLUENCE ON SURVIVAL</th>
<th>INFLUENCE ON MIGRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Bacteria survive longer at low temperature</td>
<td>Generally, migration increases under saturated flow conditions</td>
</tr>
<tr>
<td>Microbial activity</td>
<td>Increased survival time in sterile soil</td>
<td>Low pH enhances bacterial retention</td>
</tr>
<tr>
<td>Moisture content</td>
<td>Greater survival time in moist soils and during times of high rainfall</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>Increased survival time in alkaline soils (pH &gt; 5) than in acid soils</td>
<td></td>
</tr>
<tr>
<td>Salt species and concentration</td>
<td></td>
<td>Generally, increasing concentration of ionic salts and increasing cation valences enhance bacterial adsorption</td>
</tr>
<tr>
<td>Soil properties</td>
<td></td>
<td>Greater bacterial migration in coarse textured soils, bacteria are retained by the clay fraction of soil</td>
</tr>
<tr>
<td>Bacterium type</td>
<td>Different bacteria vary in their susceptibility to inactivation by physical, chemical, and biological factors</td>
<td>Filtration and adsorption are affected by the physical and chemical characteristics of the bacterium</td>
</tr>
<tr>
<td>Organic matter</td>
<td>Increased survival and possible regrowth when sufficient amounts of organic matter are present</td>
<td>The accumulation of organic matter can aid in the filtration process</td>
</tr>
<tr>
<td>Hydraulic conditions</td>
<td></td>
<td>Generally, bacterial migration increases with increasing hydraulic loads and flow rates</td>
</tr>
</tbody>
</table>

21
1.2.3.3. Transport of Bacteria

In addition to the water flow through soils, the colloidal interactions of bacteria and soil solids contribute significantly to the transport behavior. These interactions are related to physical chemical processes taking place at colloid/water interface. Since most bacteria are negatively charged, they behave very much like soil colloids, such as clay minerals and organic matter. Also, bacteria may have hydrophobic properties which may enhance the sorption to hydrophobic surface functional groups of organic matter. Hence, one can expect bacteria to move most efficiently where such surface/surface interactions are minimal. This is the case for soils and aquifers with very large pores and weakly- or non-reacting solids. In particular, the mobility is large in karstic aquifers and gravel soils and aquifers. The mobility of bacteria in aquifers has been summarized by Yates and Yates (41) and is presented in Table 4.

The most important processes involved are adsorption and its kinetics, physical straining, gravitational settling, and inertial impingement (88). While for large bacteria physical straining may be the most important factor controlling the transport behavior, adsorption dominates the retention of small bacteria (48). Transport under high flow rates may enhance the mobility of bacteria due to adsorption kinetics and/or due to decreased sedimentation and straining (89, 90). In addition to the factors influencing the solution/soil partitioning, transport phenomena such as dispersion, diffusion into immobile regions, and bypass flow through macropores may control the transport behavior. Hence, the understanding of water flow through heterogeneous soils is required to predict microorganism transport. In contrast to unsaturated soils, saturated soils are fairly well understood and models are available which describe the flow phenomena well. However, models describing unsaturated water flow in natural soils do not fully reflect the underlying mechanisms controlling the flow (2). Hence, predictive models of bacterial transport are therefore limited by the accuracy of the flow model.

Experimental studies of the possible transport mechanisms of bacteria are limited to laboratory column experiments. Wollum and Cassel (91) investigated the transport
<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Medium</th>
<th>Maximum distance traveled (m)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vertical</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Horizontal</td>
<td></td>
</tr>
<tr>
<td>Bacillus steathermophilus</td>
<td>Fractured rock</td>
<td>29</td>
<td>53</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Fine sand</td>
<td>457</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Medium to coarse sand</td>
<td>21</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Alluvial gravel</td>
<td>90</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Pea gravel + sand</td>
<td>30</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Coarse gravels</td>
<td>457</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Gravel</td>
<td>920</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Sandy clay</td>
<td>15.24</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Fine to coarse sand</td>
<td>30.5</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Fine to medium sand</td>
<td>6.1</td>
<td>62</td>
</tr>
<tr>
<td>Clostridium welchii</td>
<td>Fine + medium sand</td>
<td>15.5</td>
<td>63</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Loam + sandy loam</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sand + gravel</td>
<td>10 - 12</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>Fine sandy loam</td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Fine sand</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Pebbles</td>
<td>850</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Weathered limestone</td>
<td>1000</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Stony clay + sand</td>
<td>0.91</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Stone + clay</td>
<td>0.61</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Firm clay</td>
<td>0.3</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Coarse sand + gravel</td>
<td></td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Sandy clay loam</td>
<td>2</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Sandy clay loam</td>
<td>4.3</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>Sandy loam</td>
<td>0.64</td>
<td>28</td>
</tr>
<tr>
<td>E.coli</td>
<td>Sand</td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Fine + coarse sand</td>
<td>4</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>Fine + medium sand</td>
<td>0.15</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Fine + medium sand</td>
<td>3.1</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Sand + sandy clay</td>
<td>1.5</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>Silt loam</td>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Silty clay loam</td>
<td>1.5</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Medium sandy gravel</td>
<td>125</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Fine sandy gravel with cobbles</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Silty clay loam</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Fine sand</td>
<td>0.3</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>Fine sand</td>
<td>0.3</td>
<td>70.7</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>Fine loamy sand + gravel</td>
<td>9.1</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Stony silt loam</td>
<td>900</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Fine to medium sand</td>
<td>2.4</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Gravel with sand + clay</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Saturated gravels</td>
<td>42</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Sandy clay + clay</td>
<td>0.85</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Sandy clay</td>
<td>1.2</td>
<td>84</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>Clay</td>
<td>0.1</td>
<td>85</td>
</tr>
<tr>
<td>S. typhi</td>
<td>Limestone</td>
<td>457</td>
<td>86</td>
</tr>
<tr>
<td>Streptococcus thermophilis</td>
<td>Silty clay loam</td>
<td>0.5</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Silt loam</td>
<td>5</td>
<td>74</td>
</tr>
<tr>
<td>Streptococcus zymogenes</td>
<td>Sandy gravel</td>
<td>0.15</td>
<td>15.2</td>
</tr>
</tbody>
</table>
of *Streptomyces* conidia and an unidentified bacterium in saturated sandy soils. They found that the conidia species was preferentially transported. This behavior was attributed to pore-size exclusion. Also, the amount of bacteria retained in the columns was dependent on the pore water velocity, that is, the higher the flow rate, the smaller the amount of microorganisms retained. Poresize exclusion has also been observed for macromolecules by Enfield and Bengtsson (92) and for viruses by Grondin and Gerba (93). Another study of the difference between disturbed and undisturbed soil on the transport of *E. coli* was carried out by Smith et al. (94). In one case, they found that the macropore flow accounted for most of the microorganism transport. This phenomenon was more pronounced for the undisturbed columns than for the disturbed ones. A similar study by White and White et al. (95, 96) found that *E. coli* was restricted to only 4% of the total pore volume.

1.2.4. Biorestoration of Contaminated Soils and Aquifers

The advantage of biorestorati0on over traditional pump and treat methods are the accelerated degradation of organic contaminants, the elimination of waste products, and the generally safe operation (97). In contrast to the advantages, the long-term effects of biorestoration are virtually unknown and the changes of the subsurface ecology may cause adverse effects on chemical and physical properties of the soil or aquifer. The advantages and disadvantages of bioremediation are listed in Table 5.

According to Lee et al.(97), the basic steps in an in situ biorestoration process are:

- site investigation
- free product recovery
- microbial degradation enhancement study
- system design
- operation
- monitoring.

Site investigations include the determination of the hydraulic properties, such as the hydraulic conductivity and the specific yield of the aquifer. Furthermore, the
<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can be used to treat hydrocarbons and certain organic compounds, especially water-soluble pollutants and low levels of other compounds that would be difficult to remove by other methods</td>
<td>Can be inhibited by heavy metals and some organics</td>
</tr>
<tr>
<td>Environmentally sound because it does not usually generate waste products and typically results in complete degradation of the contaminants</td>
<td>Bacteria can plug the soil and reduce circulation</td>
</tr>
<tr>
<td>Utilizes the indigenous microflora and does not introduce potentially harmful organisms</td>
<td>Introduction of nutrients could adversely affect nearby surface waters</td>
</tr>
<tr>
<td>Fast, safe, and generally economical</td>
<td>Residues may cause taste and odor problems</td>
</tr>
<tr>
<td>Treatment moves with the ground water</td>
<td>Labor and maintenance requirements may be high, especially for long-term treatment</td>
</tr>
<tr>
<td>Good for short-term treatment of organic contaminated ground water</td>
<td>Long-term effects are unknown</td>
</tr>
</tbody>
</table>

hydrogeologic characteristics and the heterogeneity of the physical and chemical properties need to be assessed. The site history and the immediate danger of drinking water contamination will determine the type of remedial action, in particular, the time elapsed since the spill, the areal extent, the toxicity of the pollutant, and the periodicity and severity of the contamination (98, 99).

1.2.4.1. Biostimulation

Biostimulation is a process in which nutrients are added to a polluted site. These nutrients support the growth of microorganisms which degrade the pollutant in the subsurface environment. Such nutrients are phosphorous, nitrogen, and oxygen. Such an approach has been taken by Raymond (100) to biorestore an aquifer contaminated with gasoline. In 1974 Raymond (101) received a patent on such a process designed to
remove hydrocarbons by using nutrients. The successful application of biostimulation has been reported for gasoline (3000 mg/L) (102), waste solvents and alkenes (3000 mg/L) (103, 104, 105), methylene chloride (20,000 mg/L) (106), ethylene glycol (4,900 mg/L) (107), isopropanol and tetrahydrofuran (950 mg/L) (106), aliphatic hydrocarbon plasticizer (2,000 mg/L) (108), and chloroform (108). The amounts of contaminant spilled ranged from 6,000 to 303,000 L of solvents and gasolines, respectively. All of the reported cases were point source pollution with a clearly defined area of contamination.

1.2.4.2. Addition of Specialized Microorganisms

While biostimulation of indigenous microorganisms has been successfully applied, the inoculation of specialized microorganisms in contaminated subsurface environments is still not a fully developed technique (97). However, Colaruotolo et al. (109) received a patent for "microbial degradation of obnoxious organic wastes into innocuous material". They isolated microorganisms which used certain organic waste materials as their food source. By purifying and enriching such cultures, the authors were able to apply them to the contaminated site in order to accelerate the degradation process. Some of the techniques to obtain organisms which degrade contaminants rapidly include genetic engineering techniques. One method includes the exposure of microorganisms to mutagens, such as UV light, nitrous oxide, or 8-azaquinonone containing subsequently isolating organisms of a desired degradative capability (97). Alternatively, recombinant DNA technology can be applied to microorganisms to tailor the degradative abilities to specific contaminations and environments (97). The latter method is associated with the assessment of potential adverse effects of the introduced genes on the environment and human health (110). In general, the survival of engineered organisms in the environment cannot be estimated prior to an in situ test. However, due to safety considerations, it will be some time before genetically engineered microorganisms may be used in the environment. Potential problems with specialty bacteria are listed in Table 6.

The seeding of soil environments with acclimated bacteria using soils of different textures has been carried out in greenhouse experiments by Wetzel et al. (111), using
TABLE 6
REASONS WHY INTRODUCED ORGANISMS FAIL TO FUNCTION IN THE ENVIRONMENT (97)

- The concentration of the compound is too low.
- The environment contains some substance or organisms that inhibit growth or activity, including predators.
- The inoculated organism uses some other organic other than the one it was selected to metabolize.
- The organic is not accessible to the organism.

Soils of different textures. While formaldehyde was not degraded in an organic soil, the treatment was successful in the upper layer of the sandy and clay soils. The treatment of aniline-contaminated clay soil with acclimated bacteria did not enhance the degradation. Another study by Daughton and Hsieh (112) emphasized the degradation of chlorinated organics and pesticides with adapted bacteria. Their experiment succeeded in sterilized soil. However, in nonsterile soil the activity of the inoculum was greatly reduced. Overall, the success of seeding soil environments varies considerably. Environmental factors, such as temperature, pH, nutrient availability, water content, concentration of the contaminant, and the presence of growth inhibitors, may determine the success of seeding methods (97). Several types of aquifer remediation which utilize adapted microorganisms are listed in Table 7. Bacteria were used in most cases in combination with nutrient addition as well as pump and treat techniques, such as clarification, aeration, and GAC. In most cases, it was not possible to determine whether the biostimulation programs or the added bacteria were the cause of accelerated degradation rates. It still needs to be established how well inoculated bacteria can degrade contaminants in aquifers.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Treatment Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylonitrile</td>
<td>Mutant bacteria added after concentrations had been reduced by air-stripping</td>
<td>113</td>
</tr>
<tr>
<td>Phenol and chlorophenol</td>
<td>Initial treatment by adsorption onto GAC followed by inoculation with mutant bacteria</td>
<td>113</td>
</tr>
<tr>
<td>Ethylene glycol and propyl acetate</td>
<td>Treatment above ground and later with specialized bacteria</td>
<td>114, 115</td>
</tr>
<tr>
<td>Dichlorobenzene, dichloromethane, and trichloroethane</td>
<td>Initial treatment with air stripping and then inoculation with a hydrocarbon-degrading bacteria</td>
<td>114, 115</td>
</tr>
<tr>
<td>Unidentified organic compounds</td>
<td>Hydrocarbon-degrading bacteria added after levels reduced by GAC and air stripping</td>
<td>116</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Commercial degrader added to above-ground treatment system formed from rail ballast</td>
<td>116</td>
</tr>
</tbody>
</table>
2. OBJECTIVES

The conducted research studies the herbicide behavior and degradation in soils as they occur in roadside environments. To assess the effectiveness of accelerated biodegradation in soils, the retention and transport of both the herbicide and the bacteria need to be studied. Once the behavior of herbicides and pesticides are known, the best method of applying adapted microorganisms can be determined. The goals are as follows:

1. To conduct a detailed literature review on biodegradation of contaminated soil.
2. To develop experimental procedures for determining the sorption, transport, and degradation of a herbicide in one and two dimensions.
3. To assess the mechanisms controlling the pollution process of roadside soils by a herbicide, using the developed experimental procedures.
4. To study the decontamination of herbicide treated soils using adapted soil microorganisms by infiltrating, injecting, or mixing bacterial suspensions into the soil.
5. To describe the experimental transport results with mathematical models.
6. To assess the feasibility, cost, and effectiveness of biodegradation of polluted roadside environments.
3. SCOPE

This study is based on the knowledge of herbicide transport in soils obtained through years of research in the area of environmental engineering, soil physics and chemistry. Therefore, it cannot be expected to exceed the current knowledge. However, this study attempts to present a comprehensive study of pollution and decontamination of herbicides in roadside soil environments. Therefore, one cannot expect the development of entirely new methods. Rather, the existing methods of analyzing herbicide and bacteria fate in soils are used to assess the feasibility of bioremediation of contaminated soils.

The experimental techniques include batch type studies of herbicide sorption, bacteria adsorption, and biodegradation in soils with different chemical composition. Based on the initial batch study, transport experiments were carried out to assess the effect of sorption of herbicides and bacteria under more realistic conditions. While different scenarios are tested in column experiments such as solute transport with and without bypass flow, the two-dimensional experiments will reflect the spatial heterogeneity as found in field soils.
4. HERBICIDE FATE

4.1. Theoretical

4.1.1. Convective-Dispersive Equilibrium Approach

It is important to know the movement of these compounds within the liquid phase in the soil. One of the processes determining the exposure pattern of these compounds is their movement within the liquid phase in the soil media. One of the sources of ground water contamination is the application of herbicides on the roadside for vegetation management. The roadside soil generally consists of highly aggregated soil particles which will enable faster movement of water. The transport of solutes through highly aggregated soils has been studied by Green et al. (117). The liquid flow was fast in the large voids between the aggregates, while equilibrium by diffusion to smaller pores was comparatively slow. The release of solute which had penetrated into the micropores was slow and this resulted in considerable tailing in the effluent curves in a displacement experiment.

Effects of diffusion, dispersion, convection, adsorption and transport of chemicals in soil have been studied. Various conceptual-mathematical models have been developed in order to describe the one-dimensional transport of chemicals in the lab by using miscible displacement experiments. The most simple model describing the one-dimensional transport process is the Convective-Dispersive equation given by,

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x}
\]  \hspace{1cm} (1)

where,

\begin{align*}
C & = \text{Solution concentration (ML}^{-3}) \\
D & = \text{Dispersion coefficient (L}^2\text{T}^{-1}) \\
t & = \text{Time (T)} \\
x & = \text{Distance (L) and,} \\
v & = \text{Average pore water velocity (LT}^{-1})
\end{align*}

31
The above equation assumes that there is

i) Steady state one dimensional flow

ii) Constant saturated soil-water content

iii) No interaction between solute and solid phase

If there is adsorption of the solute onto soil particles, the process may be described by linear isotherm,

\[ S = kC \]  \hspace{1cm} (2)

where \( k \) = Distribution coefficient (\( M^1L^2 \))

\[ \frac{\partial S}{\partial t} = k \frac{\partial C}{\partial t} \]  \hspace{1cm} (3)

Accounting for chemical interaction between chemical and solid phase when adsorption is present, Equation (1) becomes,

\[ \frac{\partial C}{\partial t} + \frac{\rho}{\theta} \frac{\partial S}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} \]  \hspace{1cm} (4)

where, \( S \) = Sorbed concentration (\( MM^{-1} \))

\( \theta \) = Volumetric water content (\( L^3L^{-3} \))

\( \rho \) = Bulk density (\( ML^{-3} \))

\[ (1 + \frac{\rho}{\theta} k) \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} \]  \hspace{1cm} (5)

\[ R = (1 + \frac{\rho}{\theta} k) \]  \hspace{1cm} (6)

\( R \) is known as the retardation factor.
\[ \frac{R \frac{\partial C}{\partial t}}{D} = \frac{\partial^2 C}{\partial x^2} - \nu \frac{\partial C}{\partial x} \quad (7) \]

Equations (1) and (7) imply that the entire soil water participates in the convective transport of the chemicals, all adsorption sites are equally accessible for the adsorption of the solute, and equilibrium is reached instantaneously.

Most of the early transport studies were based on the linear equilibrium equations (118, 119, 120, 121). Since large deviations were observed from the observed data and predicted behavior, predictions based on the equilibrium adsorption models were found to be inadequate.

Kinetic non-equilibrium models were introduced by Lindstrom and Boersma (122) and Hornsby and Davidson (123). Success of these models was generally restricted to experiments conducted at low pore water velocities (121, 123, 124).

Several studies have been conducted in order to explain the phenomenon of tailing. One group of models explains tailing on the basis of physical processes like the presence of distinct mobile and immobile water phases. The convective transport process is assumed to occur in the mobile water phase and adsorption in the immobile phase (125, 126, 127). The other group of models explains the phenomenon on the basis of chemical processes. In this model it is assumed that on one fraction of adsorption sites, adsorption is instantaneous, while on the other sites it is time dependent (27, 28).

Analytical solutions for the convective-dispersive equation exist for several sets of initial and boundary conditions. Initial condition for this study is,

\[ C(x, 0) = C_i \quad (8) \]

Two different types of upper boundary conditions can be applied for the soil column (x = 0),

1) Constant concentration boundary condition,
\[ C(0, t) = C_0 \]  \hspace{1cm} (9)

2) Constant flux boundary condition,

\[ (-D \frac{\partial C}{\partial x} + vC) \big|_{x=0} = vC_0 \]  \hspace{1cm} (10)

where \( C_0 \) = Concentration of the input solution. The lower boundary condition is

\[ \frac{\partial C}{\partial t}(\infty, t) = 0 \]  \hspace{1cm} (11)

This condition assumes the presence of a semi-infinite column. Analytical solutions based on this boundary condition when used to calculate the effluent curves from the finite columns lead to some errors. An alternate lower boundary condition can be used

\[ \frac{\partial C}{\partial t}(L, t) = 0 \]  \hspace{1cm} (12)

where \( L \) = Column length. According to this condition, there is zero concentration gradient at the lower end of the column.

In all these models, there are different parameters which have to be quantified in order to use the transport equations to simulate the solute transport. One of the techniques used to measure these parameters is to directly estimate them from observed effluent curves by fitting the model to the experimental data (126, 128, 129).

4.1.2. Nonequilibrium Approach

4.1.2.1. Two-Site Model

The adsorption term according to this model has two components, one governed by equilibrium adsorption and the other by the first order kinetic non-equilibrium adsorption. This model has been discussed by Selim et al. (27), Cameron and Klute (28), Rao et al. (129), DeCargo et al. (130), Hoffman and Rolston (131) and by Flühler
and Jury (132). This model describes that the solid phase of the soil consists of different constituents like soil minerals, organic matter, and iron and aluminum oxides and that a chemical is likely to react with different constituents at different rates. According to the model, the sorption sites are divided into two fractions namely, "Type 1" and "Type 2". Adsorption on "Type 1" sites is said to be instantaneous and on "Type 2" sites it is time dependent. At equilibrium, adsorption on both the types of sorption sites is said to be described by linear equations,

$$S_1 = K_1 C \quad S_2 = K_2 C$$

$$= FkC \quad = (1-F) kC$$

(13)

where subscripts 1 and 2 represent type 1 and type 2 sites respectively and $F$ is the fraction of all sites occupied by type 1 sorption sites. Total adsorption at equilibrium is equal to

$$S = (S_1 + S_2) = kC$$

(14)

Since type 1 sites are always at equilibrium,

$$S_1 = FkC$$

(15)

$$\frac{\partial S_1}{\partial t} = Fk \frac{\partial C}{\partial t}$$

(16)

The adsorption rate for the type 2 kinetic non-equilibrium sites is given by a linear and reversible first order equation,

$$\frac{\partial S_2}{\partial t} = \alpha (k_2 C - S_2)$$

(17)

where $\alpha$ is the first order rate co-efficient.

Substituting for $\partial S/\partial t$ in the convective-dispersive equation, we have,
\[
\frac{\partial C}{\partial t} + \frac{\rho}{\theta} \frac{\partial S}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - \nu \frac{\partial C}{\partial x}
\]

\[S = S_1 + S_2\]  \hspace{1cm} (18)

\[
\frac{\partial S}{\partial t} = \frac{\partial S_1}{\partial t} + \frac{\partial S_2}{\partial t}
\]

\[(1 + \frac{\rho}{\theta} F k) \frac{\partial C}{\partial t} + \frac{\rho}{\theta} \frac{\partial S_2}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - \nu \frac{\partial C}{\partial x}\]  \hspace{1cm} (19)

where,

\[
\frac{\partial S_2}{\partial t} = \alpha (k_2 C - S_2)
\]

\[= \alpha [(1-F)kC - S_2] \]  \hspace{1cm} (20)

The following dimensionless parameters have been derived for the two-site model:

\[P = \frac{\nu L}{D}\]  \hspace{1cm} \[R = 1 + (\frac{\rho}{\theta} k)\]  \hspace{1cm} (21)

\[\beta = \frac{\theta + \rho k}{\theta + \rho k}\]  \hspace{1cm} \[\omega = \frac{\alpha(1-\beta)RL}{\nu}\]  \hspace{1cm} (22)

\[C_1 = \frac{C - C_i}{C_0 - C_i}\]  \hspace{1cm} \[C_2 = \frac{S_2 - (1-F)kC_i}{(1-F)k(C_0 - C_i)}\]  \hspace{1cm} (23)

Substituting the above dimensionless parameters in Equation (19) we have
\[ \beta R \frac{\partial C_1}{\partial T} + (1-\beta)R \frac{\partial C_2}{\partial T} = \frac{1}{P} \frac{\partial^2 C_1}{\partial x^2} - \frac{\partial C_1}{\partial x} \] (24)

\[ (1-\beta)R \frac{\partial C_2}{\partial T} = \omega (C_1 - C_2) \]

### 4.1.2.2. Two Region Model

This model describes that the liquid phase of the media can be divided into "mobile" and "immobile" regions. The mobile region can be described as dynamic or macro-porous and the immobile region can be described as stagnant or micro-porous.

According to the model convective-dispersive transport is restricted to the mobile phase, while transfer of solute into and out of the immobile liquid phase is assumed to be diffusion limited. The governing equation for the two region model is given by,

\[ (\theta_m + f\rho k) \frac{\partial C_m}{\partial t} + [\theta_{im} + (1-f)\rho k] \frac{\partial C_{im}}{\partial t} = \theta_m D_m \frac{\partial^2 C_m}{\partial x^2} - q \frac{\partial C_m}{\partial x} \] (25)

\[ [\theta_{im} + (1-f)\rho k] \frac{\partial C_{im}}{\partial t} = \alpha^* (C_m - C_{im}) \]

where \( C_m \) and \( C_{im} \) are the resident concentrations in the mobile and immobile liquid phases, \( q = (v + \theta_{im}) \) and

\[ \theta = (\theta_m + \theta_{im}) \] (26)

\( \theta_m \) and \( \theta_{im} \) are the mobile and immobile volumetric water contents, respectively, and \( f \) is the fraction of sorption sites in equilibrium with mobile phase, and \( \alpha^* \) is the first order rate constant governing the rate of solute exchange between mobile and immobile regions. Equation 24 can be solved for the same initial and boundary conditions as Equations 8 - 12, with \( C \) replaced by \( C_{im} \) and the additional boundary condition.
\[ C_m(0, t) = C_m(x, 0) = C_i \] (27)

The following dimensionless parameters have been derived for the two-region model:

\[ P = \frac{v_n}{D_m} \frac{L}{D_m}, \quad T = \frac{vt}{L}, \quad R = 1 + \left( \frac{\rho}{\bar{\theta}} \right) k \] (28)

\[ \beta = \frac{\theta_m + \rho k}{\bar{\theta} + \rho k} \quad \omega = \frac{\alpha L}{\bar{\theta}_m v} \] (29)

\[ C_1 = \frac{C_m}{C_m - C_i}, \quad C_2 = \frac{C_m - C_i}{C_0 - C_i} \] (30)

\( T \) is the number of pore volumes and \( P \) is the column Peclet number. Introducing the above dimensionless parameters reduces Equation 25 to

\[ \beta R \frac{\partial C_1}{\partial T} + (1-\beta)R \frac{\partial C_2}{\partial T} = \frac{1}{P} \frac{\partial^2 C_1}{\partial x^2} - \frac{\partial C_1}{\partial x} \] (31)

\[ (1-\beta)R \frac{\partial C_2}{\partial T} = \omega (C_1 - C_2) \] (32)

It should be noted that the sorption of a solute is often described by a nonlinear isotherm and hence, the model requires modification. Generally, the Freundlich isotherm is assumed to describe nonlinearity well. Van Genuchten described a procedure by which the Freundlich equation is linearized so that the above introduced models are applicable (133). The Freundlich isotherm is written as:

\[ s = Kc^n \] (33)
where $K$ and $n$ are empirical coefficients. In order to use the linear models given above, the distribution coefficient can be approximated by (133):

$$k = Ke_0^{n-1}$$  \hspace{1cm} (34)

4.2. Materials and Methods

4.2.1. Soil Sampling and Preparation

Soil samples were taken from an area located on the Baton Rouge campus of Louisiana State University. Sampling was done after clearing all the vegetation from the top of the soil. Two horizons of soil, Ap and Bt, were sampled. Ap horizon consists basically of the topsoil after removing the vegetation. Bt horizon soil is the subsoil with less organic matter compared to Ap horizon soil. All large aggregates in the soil were broken manually and the soil of both horizons was air dried for 48 hours. The dry soil was sieved through 4.8 mm and 2 mm sieves. Soil passing through 4.8 mm sieve and retained on 2 mm sieve was termed soil aggregate. Aggregated and nonaggregated soil (passing through 2 mm sieve) were used separately for experiments. Both horizons of soil were also analyzed for organic matter, pH and exchangeable cations. The results of the analysis of soil are given in Table 8.

4.2.2. Analysis of 2,4-D

2,4-Dichlorophenoxyacetic acid (2,4-D) was used as the target organic compound for all the experiments. The procedure used for 2,4-D analysis was based on an application note written by R. Schuster et al., for Hewlett Packard (134). This note describes the method for analyzing most important phenoxy-acid herbicides in ground and drinking water. The procedure involves liquid chromatography separation using a non isocratic gradient elution using binary mixture of solvents. Solvent A consisted of 0.005
<table>
<thead>
<tr>
<th>Horizon</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>pH</th>
<th>Organic matter</th>
<th>Sum of cations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-- % --</td>
<td>cmol(+) / kg</td>
</tr>
<tr>
<td>Ap</td>
<td>13.5</td>
<td>57</td>
<td>820</td>
<td>118</td>
<td>5.65</td>
<td>3.04</td>
<td>5.25</td>
</tr>
<tr>
<td>Bt</td>
<td>15.0</td>
<td>45</td>
<td>507</td>
<td>81</td>
<td>5.15</td>
<td>0.67</td>
<td>3.40</td>
</tr>
</tbody>
</table>

M KH₂PO₄ with 0.001 % CH₃COOH and solution B of acetonitrile and methanol in 1:1 ratio, also with 0.001 % CH₃COOH. The gradient started with 15 % of B (85% A) to 50% of B (50% A) at 16 minutes, to 55% of B (45% A) to 15% of B (85% A) at 21 minutes. This gradient run was carried out in order to avoid interference between several herbicides.

Since in our case the target compound was just 2,4-D, a modified procedure developed by Tamayo (135) was used. In the Hewlett Packard application note, the peak for 2,4-D appeared approximately at 9 minutes. Hence, the percentages of the two solutions at this particular time were calculated and made as a single solvent. This new solvent consisted of 65% of A and 35% of B. An ODS Hypersil column with 5 μ particles, 200 mm long and 4 mm inner diameter was used. Flow rates of 1 ml/min were maintained. Each run time was approximately 10 minutes with 2,4-D peak appearing between 6 and 7 minutes. An UV multiple wavelength detector set at a wavelength of 230 nm and band width of 12 nm, with reference wavelength and band width 450 nm and 30 nm, respectively, was used. For 2,4-D, maximum absorbance was obtained at a wavelength of 230 nm for concentration of 30 mg/l. For accuracy any concentration above 30 mg/l was diluted correspondingly. Using the modified method, the HPLC was calibrated using different standard 2,4-D solutions of concentrations
ranging from 0.5 mg/l to 25 mg/l. All concentrations were triplicated. Calibration was repeated as recommended by the manufacturer. All samples were filtered through a 0.45 μm teflon syringe filter before a 100 μl sample was injected into the HPLC. For concentrations above 30 mg/l, the samples were suitably diluted using dilution water. Dilution solution consisted of 15% Acetonitrile and 85% water, both of HPLC grade.

4.2.3. Batch Adsorption Experiments

Solutions of 2,4-D of concentrations 10, 20, 50, 75, 100 and 150 mg/l were prepared with 0.005 M Ca(NO₃)₂ background solute. Five grams of the soil were taken in a screw cap test tube of 20 ml capacity. Different concentrations of 2,4-D were added to these test tubes to obtain an approximate soil-solution ratio of 1:1. After adding 2,4-D solution, the test tubes were shaken thoroughly for proper mixing. The tubes were then placed in a rotator and agitated for 24 hours. After mixing, the test tubes were placed in a centrifuge and the mixture was centrifuged for 8-10 minutes. The supernatant from the top was separated and was analyzed for 2,4-D. All concentrations were done in triplicates. Adsorption isotherms for both Ap and Bt horizon soils were drawn. A schematic diagram of the batch adsorption experiments is illustrated in Figure 4. Time dependent batch adsorption experiments were conducted for both Ap and Bt horizon soil. These experiments were performed to determine after what time the adsorption process would attain equilibrium. The amount of 2,4-D adsorbed was monitored every 8 hours for 48 hours. Test tubes were filled with 5 grams of soil and 2,4-D solutions of two concentrations, viz., 10 mg/l and 150 mg/l for each Ap and Bt horizon soil were added. A soil solution ratio of 1:1 by weight was maintained. Then these tubes were mixed and placed in a rotating agitator. After 8 hours both Ap and Bt horizon soils were taken out and the mixture was centrifuged. The supernatant was analyzed for 2,4-D.

4.2.4. Column Transport Experiments

For miscible displacement experiments, Kontes Chromaflex glass columns of length 15 cms and diameter 4.8 cms were used. The column packing was done in five layers with tamping each layer 20-25 times using a spatula to obtain a bulk density of
Figure 4. Schematic representation of the batch experiments to study the adsorption of 2,4-D on soil.
approximately 1 gm/cm³. The procedure was repeated several times until reproducible
density were obtained. The procedure was then standardized.

The column was first packed with soil according to the procedure mentioned
above. The soil column was then saturated with 0.005 M Ca(NO₃)₂ solution. The
saturation of the column with 0.005 M Ca(NO₃)₂ solution was carried out by applying
a constant head using a Mariotte bottle. The Ca(NO₃)₂ solution was leached through the
columns in order to maintain a uniform background condition for the transport studies
as well as to stabilize the soil aggregates. After the soil column was completely saturated
with Ca(NO₃)₂ solution, a pulse of 2,4-D of concentration approximately 50 mg/l with
0.005 M Ca(NO₃)₂ background solution was given through the column. The 2,4-D
solution was pumped through the soil column using a peristaltic pump. About six to
seven pore volumes of the 2,4-D solution were pumped through the soil column. The
effluent was collected using a fraction collector. After the 2,4-D pulse, the soil column
was leached using 0.005 M Ca(NO₃)₂ solution. The effluent was then analyzed for 2,4-D
using the HPLC. From these data, breakthrough curves for 2,4-D through the soil
column were drawn.

The experiment was conducted separately with Ap horizon aggregate and soil and
Bt horizon aggregate and soil. Two different pore water velocities were used in order
to pump 2,4-D through the column. The experimental conditions of the miscible
reagents were illustrated in Table 9.

Tracer breakthrough experiments were performed on both Ap and Bt horizon
aggregate and soil to determine the hydrodynamic dispersion. A pulse of 0.1 M CaCl₂
was pumped through the soil column and the effluent was collected using a fraction
collector. Cl⁻ ion was analyzed using a Chloride electrode with a reference electrode.
A micro-electrode was used to measure Cl⁻ even with very small amounts of sample
volumes. The experimental setup used for miscible displacement experiments is given
in Figure 5. 2,4-D breakthrough curves were obtained for aggregate and soil of both Ap
and Bt horizons.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density, $\rho$ (g cm$^{-3}$)</td>
<td>1.16</td>
<td>0.95</td>
<td>0.94</td>
<td>1.32</td>
<td>1.04</td>
</tr>
<tr>
<td>Volumetric water content, $\theta$ (cm$^3$ cm$^{-3}$)</td>
<td>0.567</td>
<td>0.589</td>
<td>0.578</td>
<td>0.502</td>
<td>0.608</td>
</tr>
<tr>
<td>Percent saturation</td>
<td>89.2</td>
<td>98.9</td>
<td>99.0</td>
<td>91.4</td>
<td>92.5</td>
</tr>
<tr>
<td>Average pore water velocity, $v$ (cm h$^{-1}$)</td>
<td>2.98</td>
<td>3.04</td>
<td>13.3</td>
<td>3.88</td>
<td>3.11</td>
</tr>
<tr>
<td>Tracer pulse ($V V_0^{-1}$)</td>
<td>2.00</td>
<td>1.81</td>
<td>2.06</td>
<td>2.09</td>
<td>1.61</td>
</tr>
<tr>
<td>2,4-D pulse ($V V_0^{-1}$)</td>
<td>8.86</td>
<td>8.09</td>
<td>9.81</td>
<td>11.2</td>
<td>8.62</td>
</tr>
</tbody>
</table>

$V =$ Volume of 2,4-D or tracer pumped, $V_0$ is equivalent to 1 pore volume for the respective experiments
Figure 5. Schematic diagram of the experimental setup used for the miscible displacement experiments.
4.2.5. Two-dimensional Transport Experiments

The two-dimensional setup was designed to monitor saturated and unsaturated flow in soils in either horizontal or vertical direction. The front, side and top view of the two-dimensional flume is presented in Figure 6. The flume consists of a 759 by 759 by 51 mm main chamber containing the soil material. To the left and right of the main chamber, removable side pieces are attached. The side pieces contain the solution and sand chamber. The solution chamber is connected to eight equally spaced valves. The purpose of the valves is to create an inlet and outlet for the percolating solution with a given gradient. The sand or filter chamber prevents washout of soil particles from the main chamber.

Soil from the Bt horizon of the Loring silt loam was packed in layers into the flume. Each layer was then tampered with a rod to obtain a well mixed soil and similar bulk density throughout the flume. The soil was packed to a height of about 70 cm. The total mass of the soil packed into the main chamber was 39 kg. Assuming a particle density of quartz (2.65 g/cm$^3$) the bulk density was 1.44 g/cm$^3$.

The soil was initially wetted by connecting all valves placed at the bottom of the flume to a Mariotte bottle. The head was always kept below the wetting front until the flume was wetted up to the top. After 48 hours, the bottom valves were closed and the valves of the left side of the flume were connected to a Mariotte bottle containing 0.005 M Ca(NO$_3$)$_2$. Then the 3 top valves of the right side of the flume were opened to create a gradient between the left and right side. The water table on the left side was about 61.3 cm measured from the bottom of the soil, in contrast to 39.1 cm of the right side. Hence a head difference of 22.2 cm was established over a length of 102.3 cm. The flume was leached for 5 d with the Ca(NO$_3$)$_2$ solution until a steady flow rate of 1.23 dm$^3$/d was reached. The piezometric head was monitored at three locations in the flow direction. The hydraulic conductivity was estimated to be 9.0 $10^{-6}$, 9.7 $10^{-6}$, 2.0 $10^{-6}$, and $1.1 \times 10^{-6}$ m/s between the points of measurement.

After the initial 5 d of leaching, a 0.1 M CaCl$_2$ solution was introduced into the flume for about 10.5 d. After that, the 0.005 M Ca(NO$_3$)$_2$ solution was used to leach the chloride out of the flume. Samples of about 0.5 ml were extracted from the sampling
Figure 6. Schematic front, side, and top view of the two-dimensional flow set up.
ports with a vacuum pump operated daily at about 1 bar suction. The samples were then analyzed with a chloride ion selective micro-electrode. Chloride was monitored for 45 d after the chloride was initially introduced.

Once the chloride leaching was near completion, a 50 mg/L 2,4-D solution in 0.005 M Ca(NO₃)₂ was introduced at the same flow rate. Samples were taken daily and analyzed for 2,4-D using the same procedure described previously.

4.2.6. Parameter Estimation

The use of the Two-Site/Two-Region model requires at least three parameters which are difficult to measure independently: the dispersion coefficient (D), the dimensionless rate coefficient ω, and the dimensionless parameter β, which combines the mobile and the total water content (θₘ, θ) with the mass fraction of the solid phase in direct contact with the mobile liquid (f). In order to get an estimate of the immobile water content, aggregates of the Ap and the Bt horizon were placed on a stainless steel screen. The screen was then placed on the filter paper which was immersed in water, so that the screen was level with the water surface. The aggregates were wetted approximately 24 hours and subsequently dried at a temperature of 105°C for 24 hours.

As described earlier (Equation 21) the dimensionless parameter ω is equal to αL/θₘv. According to van Genuchten and Wierenga (126, 136) and Gaudet et al. (128), the value of the mass transfer coefficient α was not a constant and varied with pore water velocity. In order to determine the mass transfer coefficient (α), the method introduced by Rao et al. was used (137, 138). The technique is explained as follows: Data of the diffusion of the solute out of porous ceramic spheres were measured and analyzed to determine the independent estimates of the input parameters required for simulation models to describe the solute transport in aggregated porous media with distinct mobile and immobile water regions. In this method the experimental results were analyzed using two mathematical models. The first model is based on Fick's second law of diffusion written in spherical coordinates. In the second model, the rate of solute transfer into and out of the porous spheres was assumed to be proportional to the difference in solute concentration inside and outside the spheres.
Model 1:

According to model 1, the rate of solute transfer is described by Fick’s second law written in spherical coordinates,

\[
\frac{\partial C_A(r,t)}{\partial t} = D_0 \left[ \frac{\partial^2 C_A}{\partial r^2} + \frac{2}{r} \frac{\partial C_A}{\partial r} \right]
\]  

where \( C_A \) = Concentration of the solute within the sphere and \( D_0 \) is the diffusion coefficient. This equation has been solved by Crank for the average concentration of the solute within the sphere \( (C_A) \) and the external solute concentration \( (C_{M}) \) (139).

Model 2:

In this model, the time-rate of solute transfer into and out of the porous media was assumed to be proportional to the difference between the solute concentration inside and outside the spheres. This can be expressed as

\[
\theta_A \frac{\partial C_A(t)}{\partial t} = - \alpha (C_A - C_{M})
\]

where,

\[
\theta_A = \left\{ \frac{V_A}{V_{sp} + V_{ext}} \right\} \\
\theta_M = \left\{ \frac{V_{ext}}{V_{sp} + V_{ext}} \right\} \\
V_{sp} = \frac{4}{3} \pi a^3 N
\]

\( V_A \) = total solution within the spheres, \( V_{ext} \) = total solution external to the spheres and \( V_{sp} \) = total volume occupied by the spheres, \( \theta_A \) and \( \theta_M \) = volumetric water contents within the spheres and external to the spheres respectively and \( N \) = number of spheres.

By the conservation of mass,
\[ \theta_A \frac{\partial C_A}{\partial t} + \theta_M \frac{\partial C_M}{\partial t} = 0 \]  

(38)

From the above equations the expression for the time-rate change of the concentration of the solute external to the sphere can be written as

\[ \theta_M \frac{\partial C_M}{\partial t} = \alpha \left( C_A - C_M \right) \]  

(39)

Van Genuchten and Wierenga (126, 136) and Gaudet et al. (128) used equation 36 to describe the transfer of solute between the mobile and immobile water regions in the miscible displacement experiments. It was observed that the parameter \( \alpha \) is not constant and its value varied with average pore water velocity. Rao et al. (137) derived an expression relating \( \alpha \) and the physical constants of the system by comparing Model 2 with Model 1.

From equation 36,

\[ \alpha = -\frac{\theta_A \left( \frac{\partial C_A}{\partial t} \right)}{[C_A(t) - C_M(t)]} \]  

(40)

In order to determine the value of \( \alpha \), expressions for \( C_A \) and \( C_M \) from model 1 are substituted in the above equation and the following equation is derived,

\[ [C_A(t) - C_M(t)] = C_{A*} \sigma_2 \]  

(41)

\[ \frac{\partial C_A}{\partial t} = \frac{\phi C_{A*} D_o \sigma_1}{a^2} \]  

(42)

where \( \sigma_1 \) and \( \sigma_2 \) are two infinite series. From the above two expressions, the value of \( \alpha \) can be expressed as given on the next page.
\[ \alpha = \frac{D_e \phi \theta_A}{a^2} \frac{\sigma_1}{\sigma_2} \]  

(43)

Several variables from the above expression can be combined to get two nondimensional variables \( \alpha^* \) and \( t^* \),

\[ \alpha^* = \frac{\alpha a^2}{D_e \theta_A} \quad t^* = \frac{D_e t}{a^2} \]  

(44)

In the above expression for \( t^* \), the mean column residence time was chosen as the time period over which \( \alpha \) was calculated using \( t = (L/v_m) \) where \( v_m \) is the average pore water velocity in the macroporous (mobile) region. Thus by knowing the values of \( D_e, a, \theta_A, \phi \) and \( t \) the value of \( \alpha \) was calculated.

The computer program CXTFIT was used to determine the parameters. The program basically uses the non-linear least squares inversion method to identify the parameters in one-dimensional solute transport models (140). Of the several models discussed, the most commonly used equation accounting for linear adsorption is the convective-dispersive transport equation. The two-site/two-region model is described which can be applied for various non-equilibrium transport processes.

4.3. Results and Discussions

4.3.1. Adsorption Isotherms

The data from batch sorption experiments of both Ap and Bt horizons were used to obtain both linear and Freundlich isotherms. Table 10 shows the isotherm parameters along with the correlation coefficients. The correlation coefficient in the case of Ap horizon for linear isotherm is 0.999, while for Freundlich isotherm it is 0.998. So the isotherm for Ap horizon is best described by linear isotherm. The value of the distribution coefficient \( k \) is 0.572 with a standard error (SE) of 0.01.

In the case of Bt horizon soil, the correlation coefficient for the Freundlich isotherm is 0.9852 while for the linear isotherm it is 0.9818. Hence the isotherm for Bt
TABLE 10
RESULTS OF THE PARAMETER ESTIMATION OF THE ISOETHERM FIT TO THE
SORPTION DATA IN LORING SILT LOAM SOIL

<table>
<thead>
<tr>
<th>Horizon</th>
<th>$r^2$</th>
<th>RMS</th>
<th>K</th>
<th>SE</th>
<th>n</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap Linear</td>
<td>0.9994</td>
<td>1.290</td>
<td>0.5723</td>
<td>0.01000</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ap Freundsich</td>
<td>0.9984</td>
<td>0.9462</td>
<td>0.7891</td>
<td>0.1024</td>
<td>0.9249</td>
<td>0.03024</td>
</tr>
<tr>
<td>Bt Linear</td>
<td>0.9818</td>
<td>3.395</td>
<td>0.3973</td>
<td>0.02341</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Bt Freundsich</td>
<td>0.9852</td>
<td>1.895</td>
<td>1.142</td>
<td>0.2545</td>
<td>0.7474</td>
<td>0.06964</td>
</tr>
</tbody>
</table>

horizon is best described by Freundlich isotherm. The value of Freundlich parameter $K$ was obtained as equal to 1.142 with a standard error of 0.2545. The value of $n$ was 0.75 with a standard error of 0.069. The adsorption isotherms for both Ap and Bt horizons are described in Figure 7.

In the experiments, the soil solution ratio was kept at approximately 1:1 and also the Ap horizon soil had a larger quantity of organic matter. This is proved by the fact that there is significant adsorption of 2,4-D. Sorption of the pesticides on soils has been correlated with soil organic matter content \((141)\). The organic matter content in the Ap horizon soil is higher than in the Bt horizon (Table 8). This explains the reason that there is more sorption of 2,4-D in Ap horizon soil than in Bt horizon soil (Figure 7). High organic matter is associated with well structured soils and high permeability. High distribution coefficient and permeability affect each other in an opposite manner, that is, the solute is more retarded, whereas the residence time decreases with increasing pore water velocity. Also, with increasing depth the organic matter decreases and the number and size of the macropores may decrease.

4.3.2. Breakthrough Curves

4.3.2.1. Transport of Chloride

The Chloride breakthrough curves are drawn for both Ap and Bt horizon aggregated and non-aggregated soil in order to determine the hydrodynamic dispersion.
Figure 7. 2,4-D adsorption isotherms in Ap and Bt horizons of Loring silt loam obtained from batch experiments.
The tracer breakthrough curves for aggregated and non-aggregated soil for both Ap and Bt horizon soils are presented in Figure 8. The Cl breakthrough curves for the aggregated and the non-aggregated soil show a marked difference. In the case of the aggregated soil, the breakthrough appears much earlier than in the case of non-aggregated soil. Another feature is the shape of the breakthrough curve. In the case of the aggregated soil, the breakthrough curves show an asymmetric shape, whereas in the case of non-aggregated soils, the effluent concentration resembles almost plug-flow conditions. The early breakthrough and asymmetric shape of the aggregated soil curve show clear signs of bypass flow. In order to determine the parameters for the non-reactive Two Region model, the chloride data were fitted using the CXTFIT program. The parameters obtained from the fit, along with the standard error of estimates and the corresponding correlation coefficients, are shown in Table 11. In order to avoid meaningless parameter values, the dimensionless rate coefficient was approximated using the method described by Rao et al. (137, 138) as explained in section 4.2.6. For the parameter fit, the average radius of the aggregates was taken as 0.17 cm and the tortuosity factor as 0.4 (142). For these assumptions, the transfer coefficient ranged from 0.9 to 1.2 h\(^{-1}\). The corresponding dimensionless parameter \(\omega\) and \(\beta\) are listed in Table 11. The dimensionless parameter \(\beta\) equals the ratio of the mobile to total water content (\(\theta_m/\theta\)).

At least three parameters are necessary to determine the transport behavior of the solutes using the Two-Region model. The approximations of the dimensionless rate coefficient \(\omega\) and the dimensionless parameter \(\beta\) were kept constant during parameter estimation, so that the dispersion coefficient alone would be optimized (even though the correlation coefficient of all the parameter optimization of the same column are very similar). However, there was a significant change in some of the parameters when they were incorporated into optimization. These parameters are shown in Table 11, which shows drastic changes of the parameters in the case of Ap horizon soil (Ap 1) with the slow velocity run. The dispersion coefficient increased from 0.01 cm\(^2\)h\(^{-1}\) to 12.47 cm\(^2\)h\(^{-1}\) and \(\beta\) increased from 0.3 to 0.91. Since this drastic change in the parameters was not observed in the case of the fast velocity breakthrough experiment, this behavior may be attributed to data scatter. In this case, dispersion coefficient varied from 64.94 to 80.32
Figure 8. Cl⁻ breakthrough curves in the soil columns with samples from Ap and Bt horizon of the Loring silt loam with and without aggregates.
<table>
<thead>
<tr>
<th>Experiments</th>
<th>$r^2$</th>
<th>D</th>
<th>SE</th>
<th>$\beta$</th>
<th>SE*</th>
<th>$\omega$</th>
<th>SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap - soil</td>
<td>0.9831</td>
<td>0.2375</td>
<td>0.06270</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ap-aggregates(1)</td>
<td>0.9615</td>
<td>0.001</td>
<td>2.167</td>
<td>0.3</td>
<td>-</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>Ap-aggregates(1)</td>
<td>0.9797</td>
<td>12.47</td>
<td>3.034</td>
<td>0.9152</td>
<td>0.08488</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>Ap-aggregates(1)</td>
<td>0.9685</td>
<td>8.629</td>
<td>1.456</td>
<td>0.7663</td>
<td>0.03376</td>
<td>1135.0</td>
<td>183.5</td>
</tr>
<tr>
<td>Ap-aggregates(2)</td>
<td>0.9820</td>
<td>66.48</td>
<td>11.53</td>
<td>0.3</td>
<td>-</td>
<td>9.0</td>
<td>-</td>
</tr>
<tr>
<td>Ap-aggregates(2)</td>
<td>0.9841</td>
<td>80.32</td>
<td>10.99</td>
<td>0.8294</td>
<td>0.01985</td>
<td>9.0</td>
<td>-</td>
</tr>
<tr>
<td>Ap-aggregates(2)</td>
<td>0.9838</td>
<td>64.94</td>
<td>8.838</td>
<td>0.7879</td>
<td>0.03016</td>
<td>26.97</td>
<td>80.25</td>
</tr>
<tr>
<td>Bt-soil</td>
<td>0.9957</td>
<td>0.4714</td>
<td>0.05804</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bt-aggregates</td>
<td>0.9699</td>
<td>0.2502</td>
<td>1.867</td>
<td>0.3</td>
<td>-</td>
<td>1.8</td>
<td>-</td>
</tr>
<tr>
<td>Bt-aggregates</td>
<td>0.9860</td>
<td>0.5407</td>
<td>0.8325</td>
<td>0.4576</td>
<td>0.03481</td>
<td>1.8</td>
<td>-</td>
</tr>
<tr>
<td>Bt-aggregates</td>
<td>0.9914</td>
<td>2.179</td>
<td>0.9108</td>
<td>0.6299</td>
<td>0.04448</td>
<td>0.5914</td>
<td>0.1592</td>
</tr>
</tbody>
</table>

* Blank entries for the standard errors (SE) of either $\beta$ or $\omega$ indicates that the parameters were estimated independently  
† Blank entries for $\beta$ and $\omega$ indicate that the convective-dispersive equation was fitted instead of Two-region model
cm²h⁻¹. Even though the model is physically meaningful, the parameters did not seem to behave independently during the optimization process. Also, when all the three parameters were optimized, the ratio of the mobile to total water content seemed to be overestimated. Nevertheless, the parameters can be viewed as empirical coefficients which describe the mixing behavior of the non-reactive solutes in soil columns.

4.3.2.2. Transport of 2,4-D

In order to predict the pesticide transport, it is assumed that the local equilibrium is valid so that simple retardation factor R describes the chemical process. By applying the physical parameters obtained from the tracer breakthrough curves, the reactive 2,4-D transport is predicted. The 2,4-D breakthrough curves for aggregated and non-aggregated Ap horizon soils are presented in Figure 9. Even though the shape of the breakthrough curves for Ap horizon (both aggregated and non-aggregated soil) resembles closely the experimental data, the curve is more retarded, so the retardation coefficient determined from the batch experiment seems to overestimate the retardation. The 2,4-D breakthrough curve for Bt horizon is presented in Figure 10. In the case of Bt horizon, the prediction shows an excellent fit with the experimental data. This difference in the prediction may be due to the difference in the organic content in the Ap and Bt horizons. One of the miscible displacement column runs was interrupted for 48 hours in order to determine whether kinetics is involved in the process. Figure 11 describes the 2,4-D breakthrough run with 48 hours interruption in Ap horizon aggregated soil. Even after the interruption, no concentration jump is observed. From this we can assume that local equilibrium has been attained after at least seven pore volumes.

The 2,4-D breakthrough curves in non-aggregate soils (with particle size below 2 mm in diameter) show more tailing than the predicted ones. For aggregated soils of the Ap horizon, we see this effect observed to a lesser extent. In order to eliminate the non-linearity as the cause for this phenomenon, the convective-dispersive equation with the Freundlich isotherm was numerically solved for the Bt horizon. Figure 11 indicates that the tailing is more pronounced but deviates from the experimental data. Also, the breakthrough front is steeper and the deviation from the data points increased compared to the analytical solution of the linear convective-dispersive equation.
Figure 9. 2,4-D breakthrough curves in the soil columns containing soil and aggregates of the Ap horizon of the Loring silt loam.
Figure 10. 2,4-D breakthrough curves in the soil columns containing soil and aggregates of the Bt horizon of the Loring silt loam.
Figure 11. 2,4-D breakthrough curves in the soil column containing aggregates of the Ap horizon of the Loring silt loam, obtained at high pore water velocity with a flow interruption.
In order to ensure the linear equilibrium sorption cannot describe the data adequately, the transport parameter from the Two-Region model and the convective-dispersive equation were used to optimize the retardation factor \( R \) for all pesticide breakthroughs. The fitted curves for the pesticides are shown as dashed lines in Figures 9 through 11. The curves are shifted slightly towards the origin. This behavior can be attributed to the determination of the retardation coefficient from the transport data. Table 12 shows that the retardation coefficients determined from the transport data are approximately 14 to 36 \% lower than the ones determined from the batch experiments. But in the case of aggregated Bt horizon, the retardation factor determined from the transport data is higher than the one determined from the batch experiment data. However, the fitted and the predicted breakthrough curves for the aggregated Bt horizon soil show great similarities (Figure 11). This kind of behavior shows that for aggregated Bt horizon, the physical flow regime dominates the overall effluent concentration and the retardation factor is relatively insensitive towards the breakthrough.

The Two-Region model seems to adequately describe the non-reactive tracer transport. For the pesticide data, possible mechanisms explaining the deviations of the modeled and the experimental data are to be addressed. Since both linear and nonlinear isotherms cannot explain these findings, nonequilibrium processes are most likely to occur and these might explain the behavior. Also, the higher organic content in the Ap horizon may cause the difference between the aggregated Ap and Bt horizons. Hence, the higher organic contents seem to exaggerate the observed effects. This may be attributed to the intra-organic matter diffusion as reviewed by Brusseau and Rao (143). Soil organic matter can be pictured as a three-dimensional network of randomly oriented polymer chains. Humic matter in the soil is pictured as membrane-like structures (144, 145). As these membranes are porous, the existence of intra-organic diffusion is possible (143). The intra-organic matter diffusion is that the organic matter consists of polymeric materials so that the solutes can diffuse through a mesh-like structure of organic matter. Hence, the diffusion of a molecule of size in the order of magnitude of the mesh should diffuse considerably slower compared to intra-particle diffusion.
TABLE 12
PARAMETER ESTIMATES OF THE RETARDATION FACTOR FOR THE MISCIBLE DISPLACEMENT EXPERIMENTS COMPARED TO THE RETARDATION FACTOR DETERMINED FROM THE BATCH EXPERIMENTS

<table>
<thead>
<tr>
<th>Experiments</th>
<th>$r^2$</th>
<th>R</th>
<th>SE</th>
<th>$R^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap-soil</td>
<td>0.9704</td>
<td>1.806</td>
<td>0.03079</td>
<td>2.171</td>
</tr>
<tr>
<td>Ap-aggregates(1)</td>
<td>0.9719</td>
<td>1.264</td>
<td>0.08014</td>
<td>1.922</td>
</tr>
<tr>
<td>Ap-aggregates(2)</td>
<td>0.9895</td>
<td>1.203</td>
<td>0.05963</td>
<td>1.928</td>
</tr>
<tr>
<td>Bt-soil</td>
<td>0.9974</td>
<td>1.531</td>
<td>0.02265</td>
<td>2.118</td>
</tr>
<tr>
<td>Bt-aggregates</td>
<td>0.9688</td>
<td>2.064</td>
<td>0.04157</td>
<td>1.725</td>
</tr>
</tbody>
</table>

* Retardation factor determined from the batch experiments.

The data obtained during the miscible displacement experiments were reproducible. Two columns were packed with Ap horizon aggregate and all the experimental conditions were maintained the same. Figure 12 shows the effluent 2,4-D concentration with pore volumes for the Ap horizon.

4.3.3. Two-dimensional Transport
4.3.3.1. Transport of Chloride

The transport of chloride through the soil flume is presented as contour maps in Figures 13 through 24. After the solution chamber of the left side of the flume was filled with the chloride solution, the solute was transported preferentially to the right side at the bottom. The contour maps are based on the measurement of chloride samples from the ports. Hence, one can only assume that the measured concentration represents a small volume of the soil flume. The isolines were drawn by interpolating the values of the concentration of the four next neighbors with a weighting function of $1/c^3$. In the
Figure 12. 2,4-D breakthrough curves in Ap horizon aggregate, showing data reproducibility
Figure 13. Contour map of relative chloride concentration in the flume after 3 days of leaching.
Figure 14. Contour map of relative chloride concentration in the flume after 5 days of leaching.
Figure 15. Contour map of relative chloride concentration in the flume after 7 days of leaching.
Figure 16. Contour map of relative chloride concentration in the flume after 11 days of leaching.
Figure 17. Contour map of relative chloride concentration in the flume after 13 days of leaching.
Figure 18.  Contour map of relative chloride concentration in the flume after 17 days of leaching.
Figure 19. Contour map of relative chloride concentration in the flume after 22 days of leaching.
Figure 20. Contour map of relative chloride concentration in the flume after 26 days of leaching.
Figure 21. Contour map of relative chloride concentration in the flume after 32 days of leaching.
Figure 22. Contour map of relative chloride concentration in the flume after 36 days of leaching.
Figure 23. Contour map of relative chloride concentration in the flume after 40 days of leaching.
Figure 24. Contour map of relative chloride concentration in the flume after 45 days of leaching.
case where the solution may flow through large pores or cracks, the higher chloride concentration remains undetected for the sample ports which are not located in the very close vicinity of the preferential flow path. Hence, the contours in Figure 13 show isolated areas with increased concentrations. The pattern clearly shows the variability of the flow through a two-dimensional system as it may occur in nature.

Figures 14 to 16 show that first the left side (coordinates (0,0) to (20,20)) and the lower right corner (coordinates (50,20) to (76,0)) are saturated with the chloride. The center part of the flume seems to consist of nearly stagnant water which limits the chloride transport to very slow flow rates. After the pulse ends and Ca(NO₃)₂ is used to leach the chloride out of the flume, an isolated plume moves through the upper part of the flume (Figures 17 to 19). After 26 days of leaching, the nearly stagnant zone contains most of the chloride (Figure 20). This is even more pronounced at 32, 36, 40, and 45 days after the beginning of the experiment (Figures 21 to 24).

Clearly, the chloride tracer test reveals the heterogeneity of the soil flume. The importance of these findings relates to the potential pollution of groundwater by preferential flow as well as problems associate with cleanup measures. The nearly stagnant areas in a soil system may only be polluted after prolonged exposure to herbicides. Hence, cleanup measures should be taken as soon as possible after a spill. Also, in situ decontamination may be lengthy and difficult after long periods of time have passed since the contamination event.

4.3.3.2. Transport of 2,4-D

In contrast to chloride, 2,4-D is a reactive solute and is therefore retarded. As a result, one may expect different behavior in the flume. However, when comparing the 2,4-D contour map after 5 days of leaching (Figure 25) with that of the chloride (Figure 14) the shape and distribution of the solutes are similar. For this reason, the solute mixing rather than the sorption processes, seems to control the transport of 2,4-D. In contrast to the chloride pulse, 2,4-D was leached through the flume as a step function in order to saturate the flume with the herbicide. Figures 26 to 28 clearly show that the 2,4-D accumulates in the same pattern as chloride did after 8, 15, and 23 days of leaching. By day 71 the flume is completely saturated with 2,4-D (Figure 29 and 30).
Figure 25. Contour map of relative 2,5-D concentration in the flume after 5 days of leaching.
Figure 26. Contour map of relative 2,5-D concentration in the flume after 8 days of leaching.
Figure 27. Contour map of relative 2,5-D concentration in the flume after 15 days of leaching.
Figure 28. Contour map of relative 2,5-D concentration in the flume after 23 days of leaching.
Figure 29. Contour map of relative 2,5-D concentration in the flume after 71 days of leaching.
Figure 30. Contour map of relative 2,5-D concentration in the flume after 78 days of leaching.
The upper isolines in Figures 29 and 30 are caused by the water table. Since no soil solution samples were taken from the unsaturated zone, the isolines are the hypothetical distribution of 2,4-D in transition from water saturated to unsaturated soil. The flume was filled with 2,4-D in order to conduct biodegradation experiments described later.
5. BACTERIA FATE

5.1. Theory and Modeling

5.1.1. Governing Equations

The governing equations for the transport and retention of microorganisms can be obtained from the macroscopic conservation of mass for microbial particles in porous media. The capture of microbial particles from water passing through soil is the result of simultaneous action of shearing and viscous forces along with other forces that act between the particles and the collector (146). The removal mechanisms for bacteria in the porous media can be conceptualized as similar in nature to those observed for the filtration mechanisms. A review of the filtration theory (147, 148, 149) suggests that the rate of deposition of bacteria can be expressed by a kinetic equation:

\[ R_a = k_c (\theta C) - k_d (\rho \sigma) \]  \hspace{1cm} (45)

Where,

- \( R_a \) = rate of deposition of microbial particles per unit volume of soil (M/L^3T),
- \( k_c \) = clogging rate constant takes into account screening and adsorption phenomena (1/T),
- \( \theta \) = effective porosity, i.e., volume occupied by the flowing suspension per unit of the total volume (L^3/L^3),
- \( C \) = concentration of suspended microbial particles per unit volume of flowing suspension (M/L^3),
- \( k_d \) = declogging rate constant (1/T),
- \( \rho \) = density of the microbial particles (M/L^3),
- \( \sigma \) = volume of deposited bacteria per unit volume of bulk soil (L^3/L^3),
- \( h \) = constant which has to be found experimentally (h = 1 was proposed by Mints (150) and is used here).
The first term on the right hand side of the equation is the accumulation or clogging of bacteria and is considered to be primarily due to adsorption, straining, sedimentation and interception. Other mechanisms which may influence the removal of microbes in the porous media are explained later. The second term is the detachment or declogging which is due to the breaking of bacterial clusters. It is apparent from this equation that the rate of clogging is a function of the concentration of the bacterial suspension and effective porosity of the bed, whereas, the declogging rate is a function of deposited bacterial volume. The kinetic equation assumes that these two processes are simultaneous, which may not be true in the early stage of the process.

A significant volume of research has been done on adsorption of viruses to soil surfaces, but there are only a few studies on the adsorption of bacteria. The adsorption of bacteria, like viruses, was found to follow a Freundlich isotherm when straining is absent (eg. sands) and the constants will be different for different types of sands (151). But the validity of this expression for cases where straining is present (eg. clays), is not known. In case of soils containing clay, adsorption could be an important removal mechanism (152).

A particle will be strained if the particle size is larger than the pore opening. Straining results in the accumulation of particles on the soil grains thereby decreasing the pore space and hence, increasing the straining effect. The deposited bacterial volume can be estimated based on purely geometric considerations. Herzig (152) demonstrated that for bacteria the effect of straining is considerable and needs to be included in the formulation. Microbial removal by straining may not play a significant role if the mean diameter of microbes is much less than the mean diameter of the soil grains. The effect of straining may not be significant for sand medium because of the larger pores (151).

The removal of suspended particles by interception is due to the inability of particles to follow the tortuous streamlines of the fluid even though they may have the same density as the fluid. However, because of their size it is not an important mechanism for microbes.

The net rate of change of deposited microbial mass should also include the growth and death of microorganisms. If $R_{d}$ and $R_{g}$ are decay and growth terms of the deposited
microbes, respectively, then the equation for deposited particles can be written as

$$\frac{\partial (\rho \sigma)}{\partial t} = R_s - R_{sd} + R_{gd} \quad (46)$$

Substituting for $R_s$ from equation (45) in equation (46), we will get the first governing equation for the transport process.

$$\frac{\partial (\rho \sigma)}{\partial t} = k_v(\theta C) - k_q(\rho \sigma) - R_{dd} + R_{gd} \quad (47)$$

A mass balance for suspended microbial population in the control volume at the macroscopic level can be written as:

$$\frac{\partial (\theta C)}{\partial t} = -\nabla \cdot J - R_s - R_{ds} + R_{gs} \quad (48)$$

where,

- $R_{ds} = \text{rate of decay of suspended microbial particles (M/L}^3\text{T)}$
- $R_{gs} = \text{rate of growth of suspended microbial particles (M/L}^3\text{T)}$, and
- $J = \text{specific mass discharge of flowing suspension (M/L}^2\text{T)}$.

The specific mass discharge ($J$) is composed of advection and mechanical dispersion, Brownian diffusion, chemotaxis, and tumbling and movement due to sedimentation. The detailed explanation of these mechanisms is given elsewhere (97). Hence,

$$J = J_A + J_B + J_{CT} + J_{vg} \quad (49)$$

- $J_A = \text{Flux due to advection and mechanical dispersion (M/L}^2\text{T)}$,
- $J_B = \text{Flux due to Brownian diffusion (M/L}^2\text{T)}$,
- $J_{CT} = \text{Flux due to chemotaxis and tumbling (M/L}^2\text{T)}$,
- $J_{vg} = \text{Flux due to sedimentation (M/L}^2\text{T)}$.

Advection and mechanical dispersion flux is the component of movement attributed to transport by the flowing suspension. The flux term $J_A$ would contain
\[ J_A = -D_a \theta \nabla C + v_f(\theta C) \]  

(50)

where,

\[ D_a = \text{coefficient of mechanical dispersion (L}^2/\text{T}), \text{ and} \]

\[ v_f = \text{superficial longitudinal velocity of flow (L/T)}. \]

Because of their small size, bacteria rely partially on Brownian diffusion for their movement. Even though the path of the individual particles appears to be quite erratic, the average particle flux is proportional to the concentration gradient. The flux due to Brownian motion is given by (97)

\[ J_B = -D_B \theta \nabla C \]  

(51)

where, \( D_B \) is the diffusion coefficient of bacteria \((L^2/T)\). \( D_B \) can be estimated by the Stokes Einstein equation:

\[ D_B = \frac{k_B T}{3 \pi \mu_w d} \]  

(52)

where,

\[ k_B = \text{Boltzmann constant (energy per degree, ML}^2/\text{T}^2) \]

\[ T = \text{absolute temperature (°K)} \]

\[ \mu_w = \text{viscosity of the flowing fluid (M/LT)} \]

\[ d = \text{diameter of the suspended particle (L)}. \]

Chemotaxis or systematic movement of bacteria is the directed movement of bacteria toward higher concentrations of substrate. The chemotactic phenomena is a function of the substrate concentration gradient. Flux due to chemotaxis \( J_C \) can be expressed as

\[ J_C = \theta (v_m C) \]  

(53)

where, \( v_m = \text{the migration velocity (L/T)}. \)

The migration velocity is a function of relative concentration gradient and it can be formulated as a log function of the concentration gradient (154).
\[ v_m = k_m \nabla \ln C_p = \frac{k_m}{C_p} \nabla C_p \]  
\[ \text{(54)} \]

where,

\[ k_m = \text{migration rate constant or chemotactic coefficient (1/T),} \]
\[ C_p = \text{substrate concentration in the porous space (M/L}^3) \text{.} \]

However, the chemotactic motion of bacteria is frequently accompanied by another phenomenon known as tumbling, which is due to chaotic motion of bacteria. The flux due to tumbling can be formulated in the same manner as done for Brownian diffusion. However the diffusion coefficient (\(D_T\) in this case) is known as the motility coefficient and is always positive and the flux due to tumbling can be superimposed on the systematic movement, therefore the flux due to chemotaxis and tumbling is given by,

\[ J_{CT} = \theta(v_m C - D_T \nabla C) \]  
\[ \text{(55)} \]

Sedimentation occurs if the density of the suspended particles is greater than that of the fluid. Since the bacteria and viruses have densities very close to that of water, they do not tend to settle. But for some bacteria, sedimentation could be a removal mechanism \(\text{(152)}\). Settling velocity \(v_s\) can be used to quantify the significance of sedimentation and it is expressed as \(\text{(149)}\)

\[ v_s = \left[ 1 - \frac{\rho_w}{\rho} \right] \frac{m_d g}{3 \pi \mu_w d} \]  
\[ \text{(56)} \]

where,

\[ \rho = \text{density of microbial particles (M/L}^3) \text{,} \]
\[ d = \text{diameter of microbial particles (L),} \]
\[ g = \text{gravitational acceleration (L/T}^2\text{),} \]
\[ m_d = \text{mass of microbial particle (M),} \]
\[ \rho_w = \text{density of water (M/L}^3) \text{, and} \]
\[ \mu_w = \text{viscosity of water (M/LT).} \]

Therefore, flux due to settling can be expressed as
\[ J_{v_g} = \theta v_g C \] (57)

Substituting for all the flux components from Equations 50, 51, 55 and 57 in Equation 49, \( J \) will become

\[ J = -\theta D \nabla C + \theta C(v_t + v_g + v_m) \] (58)

where, \( D \), the coefficient of hydrodynamic dispersion \( (L^2/T) \), is the sum of the Brownian diffusion coefficient, coefficient of mechanical dispersion and effective diffusivity coefficient due to tumbling of bacteria, \( i.e. D = D_a + D_b + D_t \)

A mass balance for the microorganisms both in the deposited and suspended forms results in the second governing equation. This can be formulated by combining Equations (47) and (48).

\[ \frac{\partial (\theta C)}{\partial t} + \frac{\partial (\rho \sigma)}{\partial t} = -\nabla \cdot J + R_{gd} + R_{gs} - R_{dd} - R_{ds} \] (59)

Substituting for \( J \), from Equation 58 and cancelling \( R_a \), the modified form of the second governing equation is

\[ \frac{\partial (\rho \sigma)}{\partial t} + \frac{\partial (\theta C)}{\partial t} = -\nabla \cdot [-\theta D \nabla C + \theta C(v_t + v_g + v_m)] + R_{gs} + R_{gd} - R_{ds} - R_{dd} \] (60)

The decay and growth of bacteria may play significant roles in the case of long retention times for microbes in the porous media. Gerba (152) reviewed the factors that affect the survival of enteric bacteria. The decay of microorganisms is considered as a first order irreversible reaction. Assuming that the decay rate to be the same in both the adsorbed and free states the decay term becomes

\[ R_d = R_{dd} + R_{ds} = b(\theta C + \rho \sigma) \] (61)

where,

\[ R_d = \text{decay of particles in both the phases} \ (M/L^3T), \text{ and} \]

\[ b = \text{the specific decay rate} \ (1/T). \]
The specific decay rate ($b$) is typically a constant value for a particular type of bacteria and environment. Matheus and Pekdeger (155) assumed that the decay in the adsorbed state is negligible.

The growth of bacteria can be assumed to follow Monod’s equation, which describes the relationship between the concentration of a limiting substrate and the growth rate of microbes. The specific growth rate, $\mu$ (1/T), is given by

$$\mu = \frac{\mu_{\text{max}} C_F}{K_s + C_F}$$

(62)

where,

$\mu_{\text{max}}$ = maximum specific growth rate (1/T),

$K_s$ = half saturation constant (M/L³), and

$C_F$ = concentration of substrate (M/L³).

For real world situations where most of the organic matter is attached to the soil grains, the growth rate of microorganisms in the adsorbed phase may be different from that of the free phase. However, for most laboratory experiments, where there is little or no organic matter available for the growth of microorganisms, the specific growth rate is likely to be very small. In such cases, the specific growth rate of both phases can be assumed to be the same. With that assumption, the growth rate of microbial mass in the control volume is given by,

$$R_g = R_{gd} + R_{gs} = \mu (\theta C + \rho \sigma)$$

(63)

where, $R_g$ = Growth of particles in both the phases (M/L³T).

Substituting the death and growth terms from Equation 61 and 63 in Equation 60, the final form of the second governing equation will become

$$\frac{\partial (\rho \sigma)}{\partial t} + \frac{\partial (\theta C)}{\partial t} = -\nabla \cdot \left[ -\theta D \nabla C + \theta C (v_\tau + v_s + v_w) \right]$$

$$+ [\theta C + \rho \sigma] (\mu - b)$$

(64)

The third governing equation can be obtained from the mass conservation equation for the organic matter which acts as substrate for microorganisms in the control volume.
by following the similar procedure,

\[
\frac{\partial (\rho_x S_p)}{\partial t} + \frac{\partial (\theta C_p)}{\partial t} = -\nabla \cdot \left[-D_e \theta \nabla C_p + \theta v_f C_p\right] - \frac{\mu}{Y} (\theta C + \rho \sigma)
\]

(65)

where,

- \( \rho_x \) = bulk mass density of dry soil \((M/L^3)\),
- \( S_p \) = mass of adsorbed substrate per unit mass of soil particles,
- \( C_p \) = the mass of substrate per unit volume \((M/L^3)\),
- \( D_e \) = \( D_{el} + D_m \), effective diffusivity coefficient \((L^2/T)\),
- \( D_{el} \) = coefficient of mechanical dispersion of substrate \((L^2/T)\), and
- \( D_m \) = coefficient of molecular diffusion \((L^2/T)\)
- \( Y \) = true yield coefficient

Equations 47, 64 and 65 are the governing equations for the bacterial transport in porous medium. These three equations are to be solved to describe bacterial transport in a porous medium.

5.1.2. Numerical Solution

As can be seen, the governing equations are complex with a high degree of non-linearity and coupling. It is very difficult to obtain a closed form solution for the unknowns even for a one dimensional space. Numerical techniques are needed for a solution to these equations. The above governing equations can be modified to accommodate different experimental conditions. Because of the paucity of experimental data, numerical solution techniques were performed on a simplified one dimensional transport equation. In the case of saturated soils, the volumetric water content \( \theta \) is equal to the effective porosity, \((n-\sigma)\), and the velocity of flow will be a constant. If the flow is considered to be one dimensional, then the divergence term in the above equations will become a partial derivative with respect to the direction of flow. To analyze experimental conditions for the biological growth with no substrate present, the third equation need not be considered. Furthermore, in the absence of substrate, chemotactic
phenomena is absent. By incorporating all these facts and setting \( C^* = \theta C, \sigma^* = \rho \sigma, \)
\( k = \mu - b, \) in the governing equations (47 and 68) and rearranging

\[
\frac{\partial (\sigma^*)}{\partial t} = k_e C^* - k_d \sigma^* + k \sigma^* \tag{66}
\]

\[
\frac{\partial C^*}{\partial t} = D \frac{\partial^2 C^*}{\partial x^2} - u \frac{\partial C^*}{\partial x} - k_e C^* + k_d \sigma^* + k C^* \tag{67}
\]

where, \( u = v_r + v_m + v_s, \) sum of the velocities of flow, migration and sedimentation. However \( v_m \) is zero for this particular case.

The boundary and initial conditions for this problem are

\( C^* = C^*_0 \) at \( x = 0 \)
\( C^* = 0 \) at \( x = \infty \)
\( C^* = 0 \) at \( t = 0 \)
\( \sigma^* = 0 \) at \( t = 0 \)

These equations are the same as those presented by Corapcioglu and Haridas (98) for similar conditions. They obtained the analytical solution and also solved these equations numerically using the Galerkin finite element method (GFEM). In this paper, another well known numerical technique, orthogonal collocation method (OCM) is used for solving the governing equations. OCM is a combination of the collocation method and finite difference method (156). It uses the orthogonal polynomial expansions. The trial function is assumed as a series of orthogonal polynomials \( F_N(X) \) defined as

\[
F_N(X) = \sum_{j=1}^{N} C_j X^j \tag{68}
\]

The coefficients \( (C_j) \) in Equation 68 are defined by requiring that \( F_1 \) be orthogonal to \( F_0, \)
\( F_2 \) be orthogonal to both \( F_1 \) and \( F_0, \) and \( F_N \) be orthogonal to each \( F_k, \) where \( k \leq (N-1). \)

The orthogonal condition can include a weighing function \( w(X) \geq 0. \) Thus

\[
\int_{x} w(X) F_k(X) F_N(X) dx = 0. \quad k = 0, 1, 2, \ldots, N-1 \tag{69}
\]

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where a and b are the limits of integration, the polynomials satisfying Equation 68 with \( w = 1 \) are called shifted Legendre polynomials, and the roots for these polynomials are readily available in tabular form (156).

The collocation points are taken as the N roots to \( F_N(X) = 0 \), where the roots are between zero and one. The collocation points are then \( X_1 = 0.0, X_{N+2} = 1.0 \) and \( X_2 \ldots X_{N+1} \) are the interior roots. The solution for the trial function at the collocation points \( C^* (X_j) \), can be written as

\[
C^* (X_j) = \sum_{i=1}^{N+2} d_i X_j^{i-1}
\]

(70)

The first and second derivatives at the N+2 collocation points are

\[
\frac{dC^* (X_j)}{dX} = \sum_{i=1}^{N+2} d_i (i-1) X_j^{i-2}
\]

(71)

\[
\frac{d^2 C^* (X_j)}{dX^2} = \sum_{i=1}^{N+2} d_i (i-1) (i-2) X_j^{i-3}
\]

(72)

These equations can be written in matrix notation,

\[
C^* = Q d
\]

\[
\frac{dC^*}{dX} = E d
\]

\[
\frac{d^2 C^*}{dX^2} = D d
\]

(73)

where Q, E and D are (N+2) X (N+2) matrices.

The first and second derivatives can be obtained by eliminating \( d \) from Equation 73

\[
\frac{dC^*}{dX} = EQ^{-1} C^* = AC^*
\]

(74)
\[
\frac{d^2 C}{dX^2} = DQ^{-1}C = BC
\]  
(75)

When the orthogonal collocation method is applied to solve equations 66 and 67, the spatial derivatives are replaced by the following matrices

\[
\frac{\partial C}{\partial X} \bigg|_{x_i} = \sum_{j=1}^{N+2} A_{ij} C_j, \quad \frac{\partial^2 C}{\partial X^2} \bigg|_{x_i} = \sum_{j=1}^{N+2} B_{ij} C_j
\]  
(77)

Then by substituting these, Equation 66 and 67 become

\[
\frac{dC_i}{dt} = k_c C_i + (k - k_d) \pi_i
\]  
(78)

\[
\frac{dC_i}{dt} = D \sum_{j=1}^{N+2} B_{ij} C_j - u \sum_{j=1}^{N+2} A_{ij} C_j + (k - k_c) C_i + k_d \pi_i
\]  
(79)

The Runge-Kutta method was employed to integrate the above equations numerically. However, it should be noted that the above equations can also be converted to linear algebraic equations by using the Laplace transform. The solution of the orthogonal collocation method is plotted in Figure 31, with relative concentration of bacteria in suspension on the Y axis and distance along the X axis. The analytical solution and the Galerkin finite element method solution obtained by Corapcioglu and Haridas (98) are also plotted on the same figure for comparison.

To compare the solutions of the orthogonal collocation method and the Galerkin finite element method with the analytical solution, the values of the constants and parameters used were the same as those reported by Corapcioglu and Haridas (98) and are presented in Table 13. The other constants used are specific decay coefficient \(10^{-3}\) /sec, maximum specific growth \(4.2 \times 10^{-5}\) /sec and Monod half saturation constant \(2 \times 10^{-3}\) mg/l and yield coefficient of 0.04. As seen from this plot, the solution of the orthogonal collocation method (N=6) fits the analytical solution better than that of the Galerkin finite element method. Moreover, the OCM has the following advantages over the GFEM. It uses one high degree polynomial over the entire domain, whereas, GFEM

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Figure 31. Comparison of the solutions of OCM and GFEM with the analytical solution.
TABLE 13
VALUES OF THE PARAMETERS USED IN THE LITERATURE (97)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value used in the literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>dispersion coefficient (D)</td>
<td>$4.0 \times 10^{-2}$ cm$^2$/sec</td>
</tr>
<tr>
<td>clogging rate coefficient ($k_c$)</td>
<td>$6.5 \times 10^{-3}$ /sec</td>
</tr>
<tr>
<td>declogging rate coefficient ($k_d$)</td>
<td>$4.35 \times 10^{-4}$ /sec</td>
</tr>
<tr>
<td>velocity ($u$)</td>
<td>$3.0 \times 10^{-2}$ cm/sec</td>
</tr>
</tbody>
</table>

Linear polynomials are used on each element (157) and the higher order methods converge rapidly and give more accuracy than lower order methods. If the solution is symmetric, this fact can be incorporated in the trial functions, and computations can be reduced by half in the OCM. Moreover, for one dimensional partial differential equations it is easy and convenient to use OCM rather than GFEM and by increasing the number of collocation points the error can be decreased appreciably. Hence, the orthogonal collocation method is recommended for solving equations of bacterial transport through porous media.

5.1.3. Bacterial Transport through Unsaturated Porous Media

Experimental data for one special case of an unsaturated soil water flow problem have been reported in the literature (151). The governing equations for the experimental data are obtained by modifying the Equations 47, 64, and 65 to reflect the experimental conditions: (1) the flow is unsaturated and one dimensional, (2) no substrate is present, (3) effects of straining and sedimentation are negligible for the medium used and (4) the growth and decay of bacteria are neglected during the short time periods of the experiment.

Therefore, the first governing equation (Equation 47) becomes

$$\frac{\partial (\rho \sigma)}{\partial t} = k_c \theta C - k_d \rho \sigma$$  \hspace{1cm} (80)
In the absence of organic matter, chemotaxis is absent \(v_m = 0\). The effect of sedimentation is neglected because of the low settling velocity of microorganisms. Then, the second governing equation becomes

\[
\frac{\partial (\rho \sigma)}{\partial t} + \frac{\partial (\theta C)}{\partial t} = \nabla \cdot [\theta D \nabla C + v_i \theta C] + [\theta C + \rho \sigma](\mu - b) \quad (81)
\]

Considering the flow as one dimensional, the divergence will become the partial derivative with respect to the direction of flow. The above equation after rearranging the terms reduces to

\[
\frac{\partial (\theta C)}{\partial t} = \frac{\partial}{\partial X} \left[ \theta D \frac{\partial C}{\partial X} \right] - \frac{\partial v_i \theta C}{\partial X} - k_e \theta C + k_d \rho \sigma \quad (82)
\]

As there is no substrate available, the third governing equation need not be considered. Since there are two equations (Equations 80 and 82) and 3 unknowns \((\sigma, \theta \text{ and } C)\), we need additional information about the soil water content. The variation of \(\theta\) for this experiment is available \((151)\). Table 14 shows the variation of volumetric water content with Boltzmann's similarity variable \(\lambda\) which is defined as \(X/t^{1/2}\) for a diffusion type equation \((158)\). The similarity variable \(\lambda\) is used to eliminate \(X\) and \(t\) from the one dimensional transport equation.

The above governing equations 80 and 82 can be reduced to ordinary differential equations by taking \(N\) collocation points along the direction of flow. The reduced equations are

\[
\frac{d(\rho \sigma_j)}{dt} = k_e \theta_j C_j - k_d \rho \sigma_j \quad (83)
\]

\[
\frac{d(\theta C)_j}{dt} = \sum_{i=1}^{N-2} A_{ji} D(\theta_i C_i) \sum_{i=1}^{N-2} A_{ji} \theta_i C_i - v_i \sum_{i=1}^{N-2} A_{ji} \theta_i C_i - k_e \theta_j C_j + k_d \rho \sigma_j \quad (84)
\]

Initial and boundary conditions are \((151)\)

1. When \(t = 0, X > 0, C_i = 0, \sigma_i = 0, \theta_i = 0,\)
TABLE 14
VARIATION OF VOLUMETRIC WATER CONTENT WITH SIMILARITY VARIABLE (λ)

<table>
<thead>
<tr>
<th>Similarity Variable x 10^3</th>
<th>Volumetric Water Content</th>
<th>Similarity Variable x 10^3</th>
<th>Volumetric Water Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.370</td>
<td>4.5</td>
<td>0.242</td>
</tr>
<tr>
<td>0.5</td>
<td>0.340</td>
<td>5.0</td>
<td>0.233</td>
</tr>
<tr>
<td>1.0</td>
<td>0.311</td>
<td>5.5</td>
<td>0.228</td>
</tr>
<tr>
<td>1.5</td>
<td>0.292</td>
<td>6.0</td>
<td>0.218</td>
</tr>
<tr>
<td>2.0</td>
<td>0.278</td>
<td>6.5</td>
<td>0.208</td>
</tr>
<tr>
<td>2.5</td>
<td>0.265</td>
<td>7.0</td>
<td>0.190</td>
</tr>
<tr>
<td>3.0</td>
<td>0.255</td>
<td>7.5</td>
<td>0.120</td>
</tr>
<tr>
<td>3.5</td>
<td>0.250</td>
<td>8.0</td>
<td>0.015</td>
</tr>
<tr>
<td>4.0</td>
<td>0.246</td>
<td>10.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

2. when \( x = \infty \), \( C = 0 \), \( \sigma = 0 \), \( \theta = 0 \), and

3. when \( X = 0 \), \( C_1 = C \mid_{x=0} = C_0 = 1.0 \) (relative concentration is used) and \( \theta \mid_{x=0} = \theta_0 = 0.37 \)

When \( j = 1 \) \( (X = 0) \) Equation 83 reduces to

\[
\frac{d(\rho \sigma_i)}{dt} = 0.37k_c - k_d\rho \sigma_i
\]  (85)

Now taking the Laplace transform on both sides of Equation 85
\[ S \rho \sigma_i(s) = 0.37 \frac{k_c}{S} - k_d \rho \sigma_i(s) \Rightarrow \rho \sigma_i(s)(S + k_d) = 0.37 \frac{k_c}{S} \] (86)

The solution is obtained by taking the inverse Laplace transform

\[ \rho \sigma_i(t) = 0.37 \frac{k_c}{k_d} (1 - e^{-k_i t}) \] (87)

Equation 87 is a boundary condition for \( \sigma \) at \( x = 0 \) and is a function of time. Equations 83 and 84 are nonlinear ordinary differential equations. There are several techniques to solve these equations. The Runge-Kutta method is adopted here.

Equations 83 and 84 cover three sets of variables (\( \theta_i, \sigma_i, C_i \)). One can set \( C^* = \theta C \), and \( \sigma^* = \rho \sigma \), then the equations 83 and 84 reduces to

\[ \frac{d \sigma^*_j}{dt} = k_c C^*_j - k_d \sigma^*_j \] (88)

\[ \frac{d C^*_j}{dt} = \sum_{i=1}^{N+2} A_{ji} D C^*_i + \sum_{i=1}^{N+2} A_{ij} C^*_i - v_i \sum_{i=1}^{N+2} A_{ji} C^*_i - k_c C^*_j + k_d \sigma^*_j \] (89)

Equations 88 and 89 can be solved simultaneously for \( C^*_j \) and \( \sigma^*_j \). Knowing the values of \( \theta_j \) from Table 14, \( C_j = C^*_j / \theta_j \) and \( \sigma_j = \sigma^*_j / \rho \) can be calculated. The total concentration of bacteria (\( C_j \)) can be obtained by summing \( C_j \) and \( \sigma_j \).

5.1.4. Parameter Estimation and Model Verification

The above equations can be solved if the values of the parameters (\( D, k_c \) and \( k_d \)) are known. These parameters for the transport of microorganisms are not available in the literature. For the numerical solution of the governing equations, Corapcioglu and Haridas (98) used the values of the parameters from studies on the leachate movement and filtration (159, 160, 161). It should be noted that the leachate movement and filtration is somewhat different from that of transport of microorganisms through porous media.
Our approach in this research is to establish the values of the parameters $D$ (the dispersion coefficient), $k_c$ (the clogging coefficient), and $k_d$ (the declogging coefficient), which would fit the experimental data available in the literature (151). For this experiment, the flow is unsaturated and the velocity of flow through the column is not stated. However, one can use the known range of values for the hydraulic conductivity of sand ($10^{-4}$ to $10^{-1}$ cm/sec) (162), which is the medium used for the experiment and reported experimental head of water (12mm), to calculate the range of values for flow velocity by Darcy's Law. A sensitivity analysis was performed to obtain the best value of flow velocity, which minimizes the sum of squared residual between the model prediction and the experimental values. The model predictions and the experimental data are presented in Figure 32. It should be noted that the total concentration of bacteria (bacteria in both the adsorbed and free phases) is plotted as a function of a parameter defined earlier as the similarity variable ($\lambda$). The initial trial values of the parameters $D$, $k_c$ and $k_d$ are presented in Table 13. The other parameters used for the sensitivity analysis are density of bacteria (1 gm/cc) and porosity of bed (0.37). It is apparent from Figure 32 that the solution is not sensitive to flow velocity for the range of velocities used. A velocity of 0.006 cm/sec produced the minimum sum of squared residual and hence is used here.

Dispersion coefficient $D$, clogging coefficient $k_c$, and declogging coefficient $k_d$ are the three important parameters for the prediction of the microbial transport in porous media. The effect of variation of these parameters on the model prediction was analyzed by the sensitivity analysis in a manner similar to the one described above for the velocity. The effect of the variation of $D$ on the model prediction (Figure 33) suggests that the solution is sensitive to $D$ for the range of values used. The values of $D$, less than the order of $10^2$ are under predicting and the values more than the order of $10^4$ are over predicting the relative concentration of bacteria for all $\lambda$ ($\lambda = X/t^{1/2}$) values. Hence the value of $D$ is likely to be of the order of $10^2$. The least square analysis confirmed this; a $D$ value of $8.0 \times 10^2$ cm$^2$/sec produced the minimum sum of squared residual and is used for further computations. It should be noted that the value of $D$ obtained in this study is twice the $D$ value used by Corapcioglu and Haridas (28). Figure 34 shows the variation
Figure 32. Effect of varying the flow velocity on the model prediction.
Figure 33. Effect of varying the dispersion coefficient on the model prediction.
Figure 34. Effect of varying the clogging coefficient on the model prediction.
of $k_c$, the clogging coefficient on the model prediction. As can be seen from this figure, the model solution is sensitive to clogging coefficient. For all $k_c$ values, the trend of prediction changes direction at a $\lambda$ value of $1.6 \times 10^3$. The iterative search method employed to estimate the clogging coefficient yielded a $k_c$ value of $3.9 \times 10^3$ /sec. This value is about two thirds of that used by Corapcioglu and Haridas (98). Sensitivity analysis on the model prediction performed by varying the declogging coefficient is shown in Figure 35. It appears that the model solution is sensitive to declogging coefficient and for all $k_d$ values and the trend of prediction changes direction at a $\lambda$ value of $1.4 \times 10^3$. Once again the search method of minimizing the sum of squared residuals resulted in a $k_d$ value of $3.0 \times 10^3$ /sec. The $k_d$ value of this study is almost seven times that used by Corapcioglu and Haridas (98). The values of the parameters obtained in this study are summarized in Table 15.

Using the parameters from Table 15, the model is solved for $C^*$ and $\sigma^*$ by the orthogonal collocation together with the Runge-Kutta method. The results of the numerical solution using the parameters obtained in this study and the experimental data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value obtained in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>dispersion coefficient (D)</td>
<td>$8.0 \times 10^{-2}$ cm$^2$/sec</td>
</tr>
<tr>
<td>clogging rate coefficient ($k_c$)</td>
<td>$3.9 \times 10^{-3}$ /sec</td>
</tr>
<tr>
<td>declogging rate coefficient ($k_d$)</td>
<td>$3.0 \times 10^{-3}$ /sec</td>
</tr>
<tr>
<td>velocity (u)</td>
<td>$6.0 \times 10^{-3}$ cm/sec</td>
</tr>
</tbody>
</table>

from Tan et al. (151) are presented in Figure 36. The plot shows that the model under predicts the relative concentration of bacteria when $\lambda < 3.6 \times 10^3$ and over predicts when $\lambda > 3.6 \times 10^3$. 

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Figure 35. Effect of varying the declogging coefficient on the model prediction.
Figure 36. The predicted values of the relative concentration of bacteria along the experimental values.
5.2. Bacterial Transport through Soil Columns

5.2.1. Materials and Methods

5.2.1.1. Soil Preparation and Column Packing

The soil was obtained from a local site adjacent to Louisiana State University. The top 10 inches of the surface soil were removed and the soil below the top layer was excavated for experimental work. The soil was then air dried and ground in a soil grinder. This processed soil was then sieved and only the fraction passing through a 0.083mm sieve opening was used for experimental work. This soil was then mixed with fine sand at a 2:1 ratio by volume of sand to soil. This produced a synthetic soil sample with the following composition 69.3% sand, 12.3% silt, 18.3% clay, and 0.1% organic content. The sieve analysis is presented in Table 16. The soil was packed inside stainless steel columns. The columns were 2.5 inches in diameter and 12 inches in height. The column contained quarter inch threaded holes every inch down the length of the column starting one inch below the top. These holes were filled with threaded plugs. The column contained a porous stone filter at the effluent end to prevent soil wash out and a pressure gauge was located at the entrance of the column. A sketch of the complete column setup is shown in Figure 37.

The soil was serially autoclaved in shallow trays three times at 20 psi for a minimum of 30 minutes, with a one day interval between autoclavings to allow any spores to germinate. The soil was added to the column (by pouring from the top) in six increments of 251 grams each. After the addition of each soil increment, the column was packed by dropping a tamping rod 16 times from a height of approximately 5 cm above the surface of the soil. This procedure was developed in a trial and error method and the number of grams and strokes eventually chosen produced a bulk density of (1.74 g/cm³) and porosity of 34%, similar to natural soil. The remaining void space in the column, the top inch, was filled with coarse sand to prevent channeling. This technique provided a procedure which produced consistently packed columns as evidenced by the reproducible density and hydraulic conductivity (135).
TABLE 16
SOIL COMPOSITION

<table>
<thead>
<tr>
<th>Sieve Number</th>
<th>Retained (g)</th>
<th>Retained (%)</th>
<th>Cumulative (%)</th>
<th>Passing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>20</td>
<td>17.3</td>
<td>1.7</td>
<td>1.7</td>
<td>98.3</td>
</tr>
<tr>
<td>30</td>
<td>16.9</td>
<td>1.7</td>
<td>3.4</td>
<td>96.6</td>
</tr>
<tr>
<td>80</td>
<td>600.2</td>
<td>60.0</td>
<td>63.4</td>
<td>36.6</td>
</tr>
<tr>
<td>100</td>
<td>58.0</td>
<td>5.8</td>
<td>69.2</td>
<td>30.8</td>
</tr>
<tr>
<td>200</td>
<td>209.1</td>
<td>20.9</td>
<td>90.2</td>
<td>9.9</td>
</tr>
<tr>
<td>Pan</td>
<td>98.5</td>
<td>9.6</td>
<td>100</td>
<td>0.0</td>
</tr>
</tbody>
</table>

5.2.1.2. Bacterial Solution Preparation

The bacteria used in all experiments was a pure strain of a *pseudomonas* isolate (*pseudomonas DR 201*). The characteristics of this organism have been previously described (163). The bacteria were stored in a -70°C freezer in one ml aliquots of a high concentration stock solution. One ml of this solution was added to one liter of de-ionized water containing 10 grams of bacto tyrptone, 10 grams of NaCl, and 5 grams of yeast extract. This solution was placed in a shaker bath and incubated for 24 hours under mild agitation at 28°C.

The experiment utilized two types of pumping solutions at two different bacterial concentrations. One solution (high bacterial concentration) contained approximately $10^9$ cfu/ml and was prepared by centrifuging the culture solution at 10,000 rpm for 15 minutes. The bacteria were re-suspended in either sterilized 0.8% NaCl (8g/liter) solution. The second type of solution (low bacterial concentration) contained $10^7$ cfu/ml. This solution was prepared by centrifuging 18 mls of the bacterial solution in a desk top centrifuge at 14000 rpm for 15 minutes. The bacteria were re-suspended in buffered water.
Figure 37. Experimental setup for the bacterial transport study.
5.2.1.3. Adsorption Study

The ability of the microorganisms to be adsorbed to the soil was examined by placing 50 mls of bacterial suspension ($10^7$ cfu/ml) in erlenmeyer flasks. The flasks contained from 9 to 21 grams of soil. The flasks were shaken on a rotary shaker for four hours. Two mls of suspension were removed and centrifuged for two minutes at 2000 rpm in a desk top centrifuge. One ml of the supernatant was removed and enumerated.

5.2.1.4. Transport Experiments

The first set of transport experiments consisted of pumping 1.2 liters of the high bacterial concentration ($10^9$ cfu/ml) buffered water and surfactant solution through the packed columns. The pumping rate for all trials was 1.2 pore volumes per hour. The columns were pre-saturated by pumping de-ionized water into the column. The bacterial solution was pumped immediately after saturating the column. The pumping solution was stirred constantly and the container kept covered to prevent contamination. The pressure and volume pumped were recorded periodically and the effluent collected in a graduated cylinder. The pumping solution was sampled twice after 300 ml and 900 ml of pumping solution had passed through the column to determine the exact concentration of bacteria. The second set of experiments was identical to the first with the exception of passing 1.8 liters of high bacterial concentration buffered water and surfactant solution through the packed columns. The pumping solution was sampled after 300 ml and 1200 ml of pumping solution had passed through the column.

The last set of experiments consisted of passing the low bacterial concentration ($10^7$ cfu/ml) through packed columns. These runs were identical to the first set with the exception of the bacterial concentration.

5.2.5. Bacterial Enumeration

The bacterial distribution was determined by removing soil cores from each of the side ports with a corer. The corer was sterilized between each port by dipping it first in water then in methanol and flaming it. The soil cores were weighed and added to
tubes containing 9 mls of sterilized buffered water (.8% NaCl) and amended with one drop of Tween 85. This solution was shaken vigorously 25 times and allowed to settle. A serial dilution was performed for each sample and the bacteria enumerated on pour plates. The pour plate media contained 15 grams agar, 10 grams bacto tryptone, 10 grams NaCl, and 5 grams yeast extract dissolved in one liter of de-ionized water. All dilutions were plated in triplicate. The plates were incubated at 30 C° for approximately 24 hours and counted on a standard plate counter. Dilutions producing 30-300 colony forming units per plate were chosen to count although occasionally these dilutions were not available.

The pumping solution samples were enumerated by performing serial dilutions and using pour plates identical to the plates utilized for the soil enumeration, with the exception that no Tween was added to the initial dilution tube.

5.2.2. Results and Discussion

5.2.2.1 Batch Adsorption Study

The ability of the surfactant to prevent adsorption of bacteria to the soil was studied in a series of batch reactors. The concentration and volume (50mls) of the bacterial suspension was kept constant and the amount of soil varied from 0 to 21 grams. The results are presented in Figure 38. The percent adsorbed was calculated using the formula 100(Nc-Ns)/Nc where Nc is the concentration of bacteria after centrifugation of the suspension containing no soil, and Ns is the concentration of bacteria after centrifugation of the suspension amended with soil. The results indicate that the percent bacteria adsorbed on soil from the water and surfactant suspensions increases linearly with an increase in grams of soil added, up to 17 grams. Beyond 17 grams, further addition of soil does not appear to increase the microbial adsorption. In the water treatment, the lack of adsorption, when greater than 17 grams is applied to the bacterial suspension, is probably due to essentially all the bacteria being adsorbed. However, in the surfactant treatment, there is still a measurable concentration of bacteria (approximately 20%) left which do not adsorb. The linear segment of the curve for both water and surfactant solutions was observed to have a greater than 0.9 r² value. The
Figure 38. Adsorption of bacteria to soil.
TABLE 17
BACTERIAL CONCENTRATION OF PUMPING SOLUTION

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Pore Volume 300ml</th>
<th>Run</th>
<th>Sample I log(cf/ml)</th>
<th>Sample II log(cf/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW</td>
<td>4</td>
<td>1</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>7.9</td>
<td>7.8</td>
</tr>
<tr>
<td>HIGH</td>
<td>4</td>
<td>1</td>
<td>10.2</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>10.1</td>
<td>9.8</td>
</tr>
<tr>
<td>HIGH</td>
<td>6</td>
<td>1</td>
<td>9.8</td>
<td>9.9</td>
</tr>
</tbody>
</table>

The slope of both lines in Figure 38 being quite similar suggests that the rate of increase in adsorption (in %/gram) is the same for both water and surfactant.

Mozes et al., (164), observed that adsorption of hydrophilic microorganisms is essentially controlled by electrostatic interactions. The DLVO theory which deals with this type of attraction predicts that a greater difference in zeta potential for two particles of the same charge would result in a decreased chance of adsorption between the two particles (165). The zeta potential is a measure of the charge density for a given particle. The hydrophobic portion of the surfactant will bind to the bacteria, while the negative portion will increase its net negative charge. This in turn will increase its charge density and therefore its zeta potential.

Bacteria suspended in the water solution will still be negatively charged. However, the charge density should be smaller (than bacteria in surfactant solution), and thus bacteria may bind to the soil to a greater degree. This is supported by Krekeler et al., (166), who found that adsorption of microorganisms to inorganic porous materials decreased as their negative zeta potential increased. Further, Mozes et al., (164), found that negatively charged microorganisms will bind to the surface of glass, metals and plastics to a greater extent the less negative the surface.
5.2.2.2. Pumping Solution

In order to test the concentration of the pumping solutions and to examine the effects of the surfactant on the viability of the bacteria, the solution was sampled and the concentration enumerated. The concentration of all the pumping solution samples are listed in Table 17. Sample I was taken after one pore volume had been passed through the column, while sample II was taken after four pore volumes had passed through the column. This table shows that there was little difference between sample I and sample II concentrations for all pumping solutions. These data suggest little death or reproduction over the experimental time period. Furthermore, the bacterial concentration for both the high and low pumping solutions was not significantly different between the water and surfactant treatments. This result is extremely important since it implies that differences in bacterial distribution between the water and surfactant treatments are not due to differences in bacterial concentration of the pumping solutions. It also implies that prolonged exposure to the surfactant solution, at the concentration used in this experiment (1.5mM), did not affect the viability of the bacteria for the time period of the experiment. This is further evidenced by the lack of difference in bacterial concentration between the water and surfactant solution at the second sample for all runs (high and low concentration).

5.2.2.3. Effect of Bacterial Concentration on Bacterial Distribution

Two different bacterial concentrations were used in order to investigate the effects of bacterial concentration on the final distribution of the bacteria within the column. One solution (high bacterial concentration) contained approximately $10^9$ cfu/ml. The second type of solution (low bacterial concentration) contained $10^7$ cfu/ml. Four pore volumes (1200ml) of both concentrations were passed through separate columns.

The column experiments on the transportation of bacteria after passage of four pore volumes of water containing the low bacterial concentration were conducted in duplicate and the results are presented in Figure 39 and Table 18. The average distribution curve of the microbes shows a slow rise to a peak located immediately below the beginning of the soil layer (the top inch is composed of coarse sand). The
Figure 39. Bacterial distribution of low bacterial concentration after four pore volumes of treatment.
**TABLE 18**

**BACTERIAL DISTRIBUTION WATER, FOUR PORE VOLUME, LOW BACTERIAL CONCENTRATION**

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Run I</th>
<th>Run II</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log Colony Forming Units per Gram Soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.3*</td>
<td>7.9*</td>
<td>8.1*</td>
</tr>
<tr>
<td>2</td>
<td>9.0</td>
<td>8.3</td>
<td>8.7</td>
</tr>
<tr>
<td>3</td>
<td>8.7</td>
<td>7.6</td>
<td>8.3</td>
</tr>
<tr>
<td>4</td>
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<td>4.3</td>
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<td>2.7</td>
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<tr>
<td>7</td>
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<td>2.4</td>
<td>2.8</td>
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<tr>
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<td>2.2</td>
<td>2.3</td>
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<tr>
<td>9</td>
<td>1.8</td>
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</tr>
<tr>
<td>10</td>
<td>1.7</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>11</td>
<td>1.4</td>
<td>1.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* Concentration of pumping solution

Concentration of bacteria at this peak is only slightly higher than the pumping solution. The bacterial concentration drops sharply after the peak in the mid region of the column, followed by a gradual drop in concentration in the bottom third of the column. Most of the bacteria seem to be retained within the top four inches of the column with only a minor portion of the bacteria penetrating into the lower depths of the column. The continual decrease in concentration suggests that the bacterial population has not reached the saturation level in the lower depths. If the bacterial population has not reached the saturation concentration, then the bacterial population at layers below the last soil layer which is saturated should continue to decrease. This would produce a situation where,
at any point on the distribution curve which is not saturated, the concentration of bacteria is higher at a depth above the point of non-saturation and lower below this point. This is exactly what was found in the distribution.

The column experiments on the transport of bacteria after the passage of four pore volumes of water containing the high bacterial concentration were also conducted in duplicate and the results are shown in Figure 40 and Table 19. The distribution is basically uniform throughout the column with the exception of a small concentration peak at a depth of two inches. The bacterial concentration on either side of the peak is essentially the same as the concentration of the pumping solution. The uniform distribution of the bacteria on the down flow side of the peak suggests that all adsorption sites have been saturated (i.e. no new adsorption is taking place). If the adsorption sites were not saturated, the concentration of bacteria should decrease in the lower the depth of the column. However, as mentioned, there is no decrease in the distribution. Given that the distribution on either side of the peak is uniform and, as mentioned, assumed to be the saturation concentration, then the bacterial concentration peak at the top of the column is probably due to some other mechanism other than adsorption alone. This mechanism could be due to screening as noted by Bitton et al., (167).

The two bacterial distribution curves are very different and Figure 41 presents the average distributions together. The concentration of bacteria is significantly higher at all depths than the low concentration distribution. The bacterial concentration peak is below the depth at which the peak occurred in the low concentration curve. In addition, the difference between the concentration at the crest of the peak and the concentration of the pumping solution is much greater than the difference in the low concentration peak. The differences in the shape and location of the two peaks are probably due to the difference in concentration. The increased concentration of bacteria in the pumping solution would cause the bacterial concentration at the point of screening to rise at a faster rate than that observed for the low concentration treatment. This increased rate of screening would cause the difference between the bacterial concentration at the crest of the peak and the bacterial concentration of the pumping solution to increase, even though the same volume of bacterial suspension was pumped through the
Figure 40. Bacterial distribution of high bacterial concentration after four pore volumes of treatment.
## Table 19

**Bacterial Distribution Water and Surfactant Four Pore Volume High Bacterial Concentration**

<table>
<thead>
<tr>
<th>Depth (inches)</th>
<th>Run I</th>
<th>Run II</th>
<th>Run III</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log Colony Forming Units Per Gram</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10*</td>
<td>10.0*</td>
<td>10.2*</td>
<td>10.1*</td>
</tr>
<tr>
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<td>10.3</td>
<td>10.0</td>
<td>10.0</td>
<td>10.1</td>
</tr>
<tr>
<td>3</td>
<td>10.8</td>
<td>10.9</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>4</td>
<td>9.5</td>
<td>9.8</td>
<td>10.1</td>
<td>9.8</td>
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<tr>
<td>5</td>
<td>9.3</td>
<td>9.8</td>
<td>9.7</td>
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<tr>
<td>6</td>
<td>9.7</td>
<td>9.8</td>
<td>10.3</td>
<td>9.9</td>
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<tr>
<td>7</td>
<td>10.1</td>
<td>9.7</td>
<td>9.2</td>
<td>9.7</td>
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<tr>
<td>8</td>
<td>9.3</td>
<td>9.4</td>
<td>9.2</td>
<td>9.3</td>
</tr>
<tr>
<td>9</td>
<td>9.4</td>
<td>9.2</td>
<td>9.1</td>
<td>9.2</td>
</tr>
<tr>
<td>10</td>
<td>8.9</td>
<td>8.6</td>
<td>9.0</td>
<td>8.8</td>
</tr>
<tr>
<td>11</td>
<td>8.4</td>
<td>8.9</td>
<td>9.0</td>
<td>8.8</td>
</tr>
</tbody>
</table>

* Concentration in units of cfu/ml

The downward movement of the peak is harder to explain, but it may be related to the increased screening. The increased screening would cause the velocity of the solution to increase since the pores would then be smaller. This increased velocity could cause the bacteria to sheer from the point of clogging and thus move further into the column.

### 5.2.2.4. Distribution Changes Due To Increased Pumped Volume

The effect of increasing the volume of solution pumped for the same bacterial concentration (high) was examined by pumping four and six pore volumes of the high bacterial concentration solutions through columns.

The distribution curve obtained from pumping six pore volumes of high bacterial concentration suspended in water departs markedly from that observed from the passage
Figure 41. Comparison of bacterial distributions of high and low bacterial concentrations after four pore volumes of treatment.
of four pore volumes of water containing a high bacterial concentration as previously discussed. The six pore volume treatment curve is an average of two distributions found in Figure 42 and Table 20. The six pore volume bacterial distribution is fairly constant for the first three inches of the column, which is in contrast to the four pore volume distribution curve that shows a peak in this region. The bacterial concentration then makes a sharp increase to a peak level located four inches from the top of the column and one inch below the peak found in the four pore volume treatment. After the peak, the bacterial concentration decreases at a fairly constant rate, as opposed to the uniform distribution found in the four pore volume curve. The peak is also of much greater magnitude than the peak in the four pore volume treatment.

This peak, as in the four pore volume treatment, could possibly be caused by screening. The downward migration and increase in magnitude of the peak could be caused from the increased pumped volume. The increased pumped volume could have caused the dislodging of clay and fine soil particles from the upper depths, increasing the soil density at lower depths. If the soil density did increase, then a partial blockage of the pores would occur. Once the pores became blocked, the concentration of bacteria would increase at the point of blockage and less bacteria would be able to penetrate to the lower depths. The decrease in the concentration of bacteria in the lower depths is consistent with the screening phenomenon. If the pores have become clogged, it would produce a situation where only the suspending solution would be able to pass the blocked pores. Therefore, the lower depths of the column located below the clogged pores would be subjected to a solution with very low concentrations of bacteria relative to the concentration of bacteria on the soil. This would result in the desorption of bacteria from the soil to the pumping solution. The bacteria desorbed would then be washed out of the column and no new bacteria would be available to replace those removed due to the blocked pores. This would lower the bacterial concentration in the lower depths of the column.

Figure 43 is a composite graph of the average distributions for both the four and six pore volume water treatments containing the high bacterial concentration. It appears that the mechanism of bacterial retention in soil consisting of adsorption and screening

121
Figure 42. Bacterial distribution of high bacterial concentration after six pore volumes of treatment.
TABLE 20
BACTERIAL DISTRIBUTION AFTER SIX PORE VOLUMES
OF HIGH BACTERIAL CONCENTRATION

<table>
<thead>
<tr>
<th>Core</th>
<th>Run I</th>
<th>Run II</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log (cfu/gr Soil)</td>
<td>Log (cfu/gr Soil)</td>
<td>Log (cfu/gr Soil)</td>
</tr>
<tr>
<td>0</td>
<td>9.8*</td>
<td>9.8*</td>
<td>9.8*</td>
</tr>
<tr>
<td>2</td>
<td>10.1</td>
<td>10.0</td>
<td>10.1</td>
</tr>
<tr>
<td>3</td>
<td>10.4</td>
<td>10.4</td>
<td>10.4</td>
</tr>
<tr>
<td>4</td>
<td>11.7</td>
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<td>11.4</td>
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<tr>
<td>6</td>
<td>9.5</td>
<td>10.5</td>
<td>10.0</td>
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<td>7</td>
<td>9.0</td>
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<td>8.5</td>
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<td>8</td>
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<td>7.1</td>
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<tr>
<td>9</td>
<td>7.6</td>
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<td>7.3</td>
</tr>
<tr>
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<td>7.1</td>
<td>4.8</td>
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</tr>
<tr>
<td>11</td>
<td>4.8</td>
<td>3.1</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Concentration in units of cfu/ml

is the same for both the four pore volume and six pore volume high bacterial concentration treatments. However, the peak found in the six pore volume treatment is of greater magnitude and at a lower depth than the peak in the four pore volume water treatment. If the volume pumped is increased, then more bacteria are applied or pumped into the column. Therefore, more bacteria are available to become screened, increasing the magnitude of the peak and thus the degree of blockage, compared to the four pore volume peak for the same bacterial concentration.

Therefore, it appears as though pumping greater volumes of bacterial solution in the water treatments leads to an increase in the magnitude of the bacterial concentration peak found in the four pore volume, high bacterial concentration treatment. Furthermore, the level of screening is sufficient to decrease considerably the
Figure 43. Comparison of the bacterial distributions of high bacterial concentration after four and six pore volumes of treatment.
concentration of bacteria in the lower depths as opposed to the four pore volume, high bacterial treatment. In that treatment, screening did cause a peak but the level of blockage was not sufficient to reduce the concentration of bacteria below the peak.
6. BIODEGRADATION STUDIES

6.1. Materials and Methods

6.1.1. Bacterial Solution

Two strains of *Pseudomonas* bacteria have been isolated by Roy at Louisiana State University which are capable of degrading 2,4-D in aqueous media. These two isolates of *Pseudomonas* are under patent by Roy (United States Patent 4816403) (163). They are named as *Pseudomonas* DR 101 and DR 201. These isolates are gram negative, nonfermentive and motile rods. The bacteria used in all the experiments was a pure strain of *Pseudomonas* DR 201. The bacteria were first grown on L-B media. Later on, these bacteria are centrifuged and transferred onto 2,4-D media so that they get acclimatized to 2,4-D. The bacteria grown on 2,4-D media were then centrifuged. These bacteria were used to prepare the bacterial suspension. Various media were used in order to promote the growth of the microorganisms and also for microbial seed population maintenance and magnification. The composition of the various media follows:

i) Basal Salt Media (10 X):

Basal Salt Media (10 X) are a liquid source of the inorganic nutrients necessary for the microorganisms. The medium was prepared by dissolving 58.0 g of K$_2$HPO$_4$, 45.0 g of KH$_2$PO$_4$, 20.0 g of (NH$_4$)$_2$SO$_4$, 1.6 g of MgCl, 200 mg of CaCl$_2$, 20.0 mg of NaMoO$_4$ and 10.0 mg of MnCl$_2$ in 1.0 liter of de-ionized water.

1X refers to 1:10 dilution of 10X BSM with de-ionized water. The BSM used for all applications was autoclaved at 250 F at 20 psi for 20 minutes.

ii) LB (Luria-Bertani) media:

The media were used to grow the microorganisms initially before exposing them to the 2,4-D environment. LB media are prepared by dissolving 10 g of bacto-tryptone, 5 g of bacto-yeast extract and 10.0 g of NaCl in 1 liter of deionized water.

iii) LB (Luria-Bertani) Agar Media:

Microorganisms were plated in LB medium which was prepared by dissolving 15 g of agar, 10 g of bacto-tryptone, 5 g of bacto-yeast extract and 10.0 g of NaCl in 1 liter
of deionized water. The medium was autoclaved at 250 F at 20 psi for 20 minutes and allowed to cool down such that it could be poured into the plates.

iv) 2,4-D 1X media:

The media were used to grow the microorganisms which are capable of using 2,4-D as their nutrient media. One liter of 2,4-D medium was prepared by mixing the following:
1) 70 ml of 2,4-D stock solution (25 g/l)
2) 100 ml of 10X BSM (sterile)
3) 830 ml of sterile deionized water

6.1.2. Batch Experiments

The batch bio-degradation experiments were conducted using both Ap horizon aggregates and soil. Fifty grams of Ap horizon soil were taken in conical flasks with screw caps of volume of 250 ml. To these flasks different concentrations of 2,4-D solutions were added so that the soil solution ratio was approximately 1:1 by weight. Three different concentrations of 2,4-D solutions of 50 mg/l, 200 mg/l and 500 mg/l were used for the batch biodegradation studies. Five ml of 1X BSM solution were added to each flask. The concentration of the 2,4-D solution added was adjusted so that the final concentration of the 2,4-D in the flask after adding the BSM solution was 50 mg/l, 200 mg/l and 500 mg/l respectively. The pH of the solution in the flasks was noted. The flasks were placed in a shaker maintained at a constant temperature of 28 °C. The flasks were left in the shaker for 24 hours so that the adsorption of 2,4-D on the soil particles was complete. After 24 hours, a small quantity of the solution was sampled out from the flasks. This solution was centrifuged and the supernatant was separated and then analyzed for 2,4-D using the HPLC. The amount of 2,4-D left in the solution after adsorption was determined.

After 24 hours of agitation, 1 ml of the of the bacteria suspension was added to each flask. The flasks were left in the shaker with the caps not completely tightened so that there was flow of air into and out of it. After 24 hours, the solution from the flask was sampled and centrifuged. The supernatant was then analyzed for 2,4-D. All
experiments were done in duplicates. A schematic diagram of the batch biodegradation experiments is illustrated in Figure 44.

The concentration of the microorganisms used for both batch and column experiments was in the range of $10^6 - 10^7$ cfu/ml (colony forming units/ml) of the suspension.

6.1.3. Column Experiments

In order to determine what volume of the microbial suspension has to be pumped into the column so that it is completely saturated with the microorganisms, the column was packed with A-horizon soil and the bacterial suspension was pumped. The effluent bacterial concentration was monitored.

The soil column was packed with Ap horizon aggregate. Then the column was saturated with 2,4-D solution. The centrifuged bacteria were transferred to a solution of 2,4-D having the same concentration used for saturating the column. Then the bacteria suspension was pumped through the soil column and incubated for 48 hrs. After the incubation period, the 2,4-D solution of the same concentration used to saturate the column was pumped and effluent 2,4-D concentration was monitored. Effluent was collected using a fraction collector. Two different concentrations of 2,4-D solution, namely 50 mg/l and 500 mg/l, were tried in the column experiments. In the first experiment (50 mg/l 2,4-D solution) the injected microorganisms were suspended in 2,4-D solution only (no source of inorganic nutrients). In the second experiment using 500 mg/l 2,4-D solution, 8 ml of 10X BSM were added to 400 ml of the bacterial suspension in 2,4-D solution. This was done in order to determine whether the microorganisms require an additional inorganic nutrient source.

6.1.4. Two-dimensional Experiment

The two dimensional flume was used to monitor the disappearance of 2,4-D within the flume. As described previously, the flume was saturated with 2,4-D. Then, a bacterial suspension was injected into the first column of ports on the left side. The suspension had a density of $10^9$ of which 50 ml were equally distributed among the seven
Figure 44. Schematic representation of the batch biodegradation experiments.
<table>
<thead>
<tr>
<th>Soil</th>
<th>Input Conc. of 2,4-D mg/l</th>
<th>pH of the mixture of 2,4-D and soil</th>
<th>Conc. of 2,4-D remaining after adsorption, mg/l (Duplicates)</th>
<th>Final Conc of 2,4-D in the soln mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap aggregates</td>
<td>1) 50</td>
<td>6.6</td>
<td>i) 29.74</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii) 30.78</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2) 200</td>
<td>6.7</td>
<td>i) 162.46</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii) 157.43</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3) 500</td>
<td>6.6</td>
<td>i) 383.34</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii) 367.65</td>
<td>2.6</td>
</tr>
<tr>
<td>Ap soil</td>
<td>1) 50</td>
<td>5.7</td>
<td>i) 29.90</td>
<td>0</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>ii) 30.70</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2) 200</td>
<td>5.8</td>
<td>i) 145.92</td>
<td>0</td>
</tr>
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<td></td>
<td>ii) 137.83</td>
<td>0</td>
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<td>3) 500</td>
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<td>i) 376.17</td>
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<td></td>
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<td></td>
<td>ii) 349.71</td>
<td>0</td>
</tr>
</tbody>
</table>
injection ports. The 2,4-D concentration was then monitored in the downstream direction. Since the flume has been saturated with 2,4-D, any deviation from steady state needs to be interpreted as the success of the bacteria to degrade the pesticide.

6.2. Results and Discussion

6.2.1. Batch Experiments

Table 21 presents the concentrations of 2,4-D obtained after the adsorption and incubation period for initial concentrations of 50, 200 and 500 mg/l of 2,4-D, under batch conditions. The pH of the soil and 2,4-D mixture has also been reported in Table 21.

The pH of the soil has an effect on the rate of degradation of 2,4-D in soil. The pH might affect the degree of adsorption by the soil and also the activity of the microbiological population. Optimum pH range for the degradation of 2,4-D has been reported as 5 to 7. The values of pH vary between 6.5 and 7.0 in the case of A-horizon aggregate and between 5.5 and 6.0 in the case of A-horizon soil. In both cases there are no extreme values of pH which will affect the biodegradation process.

From Table 21 it is evident that the microorganisms are using 2,4-D as their nutrient source and within 24 hours all the 2,4-D present in the solution has been degraded. Except in the case of 500 mg/l of initial concentration where a residual of 2.6 mg/l of 2,4-D is present after 24 hours, in all other cases there is complete degradation. It should be noted that the temperature was maintained at 28 °C and the flasks were kept in a shaker, so that the conditions were conducive for the microorganisms to grow. From Table 21 it can be inferred that these microorganisms can be used for bio-remediation of soil contaminated by 2,4-D.

6.2.2. Column Experiments

Table 22 shows the concentration of the microorganisms in the effluent of the soil column after pumping the bacterial suspension. The input concentration was 10^7 cfu/ml. The table shows that by 1.5 pore volumes the column is saturated with the
TABLE 22
EFFLUENT BACTERIAL CONCENTRATION FROM THE SOIL COLUMN FOR DIFFERENT PORE VOLUMES OF BACTERIAL SUSPENSION

<table>
<thead>
<tr>
<th>Pore Volume of bacterial suspension pumped</th>
<th>Effluent bacterial concentration cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>$10^6$</td>
</tr>
<tr>
<td>1.5</td>
<td>$10^7$</td>
</tr>
<tr>
<td>2.0</td>
<td>$10^7$</td>
</tr>
</tbody>
</table>

microorganisms and the effluent concentration of the microorganisms is the same as the input concentration. For all column experiments, two pore volumes of the bacterial suspension were used to saturate the column with microorganisms.

Figure 45 shows the comparison of the effluent 2,4-D concentrations from the columns for an initial concentration of 50 mg/l, with 2 and 4 days of incubation period. The results from the first column experiment with an input concentration of 50 mg/l of 2,4-D and 2 and 4 days of incubation show that there is very little degradation. Figure 45 illustrates that there is not appreciable biodegradation of the input 2,4-D concentration. Possible reasons for this may be the non-availability of nutrients at the initial stages. Even though the incubation period was doubled from 2 days to 4 days, the amount of 2,4-D being degraded is almost the same. From this it can be inferred that the time duration available is not a factor which is influencing the biodegradation process.

Figure 46 shows the comparison of effluent 2,4-D concentrations from columns with two different initial concentrations of 50 and 500 mg/l with the same incubation period of two days. Also in the case of 500 mg/l of input concentration, the microbial suspension was supplemented by basal salt media. The comparison shows that even though the concentration of 2,4-D was increased tenfold and additional inorganic nutrients were available to the microorganisms, there is not much variation in the amount of 2,4-D degraded.
Figure 45. Effluent concentrations of 2,4-D after biodegradation, from the column with Ap-horizon aggregate with an input concentration of 50 mg/l and with incubation periods of 2 and 4 days.
Figure 46. Effluent concentrations of 2,4-D after biodegradation, from the column with Ap-horizon aggregate and with input concentration of 50 mg/l and 500 mg/l and an incubation period of 2 days.
The rates of microbial transformations of herbicides are influenced by many environmental factors. The individual effect of these factors may sometimes be difficult to determine as they may be interrelated (Torstensson, 1980). There are several reasons for the ineffectiveness of specialty microorganisms to degrade 2,4-D. According to Goldstein et al. (1985), some of the reasons the microorganisms do not metabolize the pollutants in the environment are: 1) the concentration of a xenobiotic may be too low to support the growth of the microorganisms 2) the microorganisms may be susceptible to predators or toxins 3) other organic matter may be used by the microorganisms in preference to the pollutants 4) movement of the microorganisms may be difficult in the soil media. The first reason can be ruled out because two different concentrations were tried, the second (500 mg/l) being ten times greater than the first. The second reason can also be ruled out as the microorganisms used are accustomed to 2,4-D environment and are, in fact, grown on 2,4-D media. Since in batch experiments the 2,4-D was completely degraded, the possibility of the microorganisms using any other organic matter in preference to 2,4-D can also be excluded (Reason 3). The fourth reason, inadequate transport of microorganisms in the soil media, might have decreased biodegradation. Another possible reason might be the non-availability of sufficient oxygen in the soil column for complete metabolic activity of the microorganisms. Since these systems typically operate aerobically, availability of oxygen might be a crucial factor (168).

Temperature has an important role to play in the microbial degradation of the chemicals. The temperatures generally accepted as optimal for the microbial activity range between 20 - 30°C. In the batch experiments, the temperature in the shaker was maintained at 28°C. It should be noted that there was no temperature control for the column experiments even though the temperatures did not go below 15°C. There is a possibility that there was some effect of the temperature on the degradation in the soil columns.

Apart from the environmental factors, sorption processes also have an influence on the biodegradation of various chemicals (169). Sorption has accounted for both increase and decrease of the biodegradation rates (170). Increase in the biodegradation
rate has been reported when the compound to be degraded has been found to be toxic to the microorganisms. In our case, since the microorganisms being used were acclimatized to the 2,4-D environment, the toxicity effect can be ruled out. It has been more often reported that the sorption decreases the biodegradation rates (171). Ogram et al. (36) and Chakraborty et al. (172) discuss that microorganisms can utilize only the dissolved substrates. Sorption can retard the degradation process by decreasing the aqueous concentration levels or by limiting biodegradation rates with small desorption rates. This will result in the desorption controlled biodegradation (173). For porous adsorbents such as aggregates, the intra-aggregate diffusion process can further contribute to the retardation of the solute transfer between the adsorption site and the bulk liquid. This will enhance the retardation of the degradation rate, since the diffusion rate depends on the solute concentration. The biodegradation of 2,4-D in batch experiments can also be explained by the collapse of aggregate due to mixing, by which the 2,4-D sorbed in the intra-aggregate pores was available to the microorganisms for degradation.

In both Figures 45 and 46, we see that the relative effluent concentration starts at 1.0. But after biodegradation it is expected to fall and then rise gradually to the value of 1.0 with the pumping of 2,4-D solution. From this it is inferred that the 2,4-D present at the top portion of the column is not being degraded. This may be due to the presence of some dead volume in the top of the column even though precautions were taken to avoid any dead volume.

6.2.3. Two-dimensional Experiment

As seen in Figure 47, the biodegradation was only partially successful. More specifically, Figure 47 shows the contours of relative 2,4-D concentration at day 78 and 81 after the beginning of 2,4-D leaching. In contrast to the previous contour maps, this map is drawn for a concentration range of 0.85 to 1.00. The upper graph (A) represents the fully saturated flume before the injection of bacteria. The 0.97 isoline runs from the upper left corner to the middle of the right side of the flume, following the location of the water table. The concentration below this contour is higher then 0.97, and thus indicates full 2,4-D saturation. The bottom graph (B) clearly shows a different pattern
Figure 47. Result of the two-dimensional biodegradation study with contours ranging from 0.85 to 1.00 relative 2,4-D concentration.
of the 0.97 isole. In fact, the shape of this line resembles the pattern observed for the chloride and 2,4-D leaching presented earlier. This indicates that the induced biodegradation is most successful in high flow regions.
7. COST FUNCTIONS OF BIOREMEDIATION USING ACCLIMATED BACTERIA

The goal of enhanced biodegradation is to clean up roadside spills quickly to minimize public exposure to health hazards. A principal advantage of biodegradation is the elimination of contaminants rather than their transfer to another area (i.e., Landfill). Moreover, inoculated microorganisms degrade contaminants much faster (by orders of magnitude) than natural soil microorganisms, which may be destroyed by the spilled chemical (174). Rapid treatment is particularly important when long treatment times would interfere with traffic or extend the plume of contamination through leaching.

It is important to distinguish between accidental spills and managed applications of herbicides along roadsides. Accidental spills involve high concentrations of herbicides over relatively small areas, whereas, deliberate applications use smaller concentrations applied over large areas. Applied herbicides are transported to rivers, as evidenced by their continual presence in the Mississippi River (175). Herbicides used on roadsides can exceed 10 percent of the amount applied to land for agriculture (175).

Specialty bacteria have a potential application in conjunction with deliberate applications of herbicides. For example, beneficial bacteria could be sprayed, simultaneously or after a lag time, with herbicides to insure their rapid biodegradation in the roadside environment, especially in areas with a high leaching or runoff potential. In the case of spills, the introduction of a bacterial seed may be required if the natural soil microorganisms have been destroyed by the spilled chemical. Even if the natural microflora are still viable, the specialty microorganisms can expedite the remediation rate. Several treatment schemes are possible, depending on the spill scenario. Table 23 (174) outlines appropriate treatment procedures for various types of spills.

For a given spill, optimal selection of cleanup methods requires comparison of the costs and benefits of technically feasible alternatives. Costs of cleanup include capital, operational, and secondary expenses. Capital requirements are the mobile equipment necessary to contain and treat a spill. Operational expenses are mainly labor and the costs of energy and nutrients. Secondary expenses are incurred due to the presence of the pollutant in the roadside
TABLE 23
EMERGENCY MANAGEMENT STRATEGIES TO MINIMIZE POLLUTION
FROM ROADSIDE CHEMICAL SPILLS

**Water Spill**
- Impound contaminated water
- Remove any bulk chemical
- Environmentally condition water (pH, nutrients, temperature)
- Seed with microorganisms
- Aerate
- Polish water as necessary (e.g., activated carbon adsorption)
- Recharge with more microorganisms or discharge as appropriate

**Land Spill**
- Contain runoff
- Collect and remove bulk chemical
- Condition soil (pH, nutrients, water, cultivation)
- Seed with microorganisms
- Continue cultivation

**Contaminated Groundwater**
- Impound water
- Recover and remove bulk chemical
- Prepare recovery wells
- Condition impoundment water (pH, nutrients)
- Seed with microorganisms
- Recirculate treated leachate to ground
- Polish treated leachate as necessary
- Discharge to receiving waters
environment. Examples of secondary costs are traffic disturbances (cost of rerouting traffic) and the cost of polluting or shutting down a public water supply.

7.1 Technical Feasibility

Optimal management of roadside spills requires a technical screening followed by an economic screening of treatment alternatives. Technical screening considers the biogeochemical properties of the spilled chemical and the roadside environment to define workable cleanup methods. A technical screening will generally indicate whether to bioremediate or remove the contaminated soil (landfill or incinerate). Once all technically feasible alternatives are selected, an economic analysis will identify the least cost treatment method. Economic screening considers all the factors related to costs (including secondary costs), benefits, and risks. There are inherent uncertainties and loosely predictable elements in both technical and economic analysis of spills. These uncertainties can be minimized by breaking up analysis of complex spill scenarios into simple components. Technical uncertainties tend to be greater for bioremedial treatment than for landfilling and incineration. For example, unlike excavation and landfilling, a bioremediation technique cannot guarantee 100 percent removal of the spilled chemical. On the other hand, excavation is unlikely to be applied to remove low residual concentration of a spilled chemical at the margins or boundaries of the contaminated region. In situ bioremediation can remEDIATE much of the residual concentration.

When deciding between bioremediation and other treatment options, the properties of the spilled chemical and the roadside environment must be quantified. The type of chemical (organic, inorganic), its concentration, and its form (dry pellets or aqueous) are important technical considerations. Biodegradation is best suited for wet spills of organic compounds such as hydrocarbons and herbicides, whereas landfilling and incineration are best for treating toxic chemicals (i.e., heavy metals) and dry spills.

The technical feasibility of using acclimated bacteria to remediate spills currently limits the number and type of potential field applications. Technical results from this study distinguish potential applications from unsuccessful ones. Success of batch biodegradation studies suggests the use of acclimated bacteria to treat soil slurries and/or pumped ground water. Successful injection of acclimated bacteria to polluted regions of an aquifer is not technically superior to
enhancing natural soil bacteria at this time. Technical problems are based on the inability to transport bacteria and electron acceptors (i.e., oxygen) to the impermeable regions of a heterogeneous aquifer.

7.2 Costs and Extent of Spills

Costs of cleaning up a herbicide spill will depend on the spatial extent of the pollution which is a function of the contaminant, the groundcover, and the soil and climatic conditions at the time of the spill. Hydrophilic (very soluble) contaminants spilled in sandy, unvegetated, dry soils near a shallow drinking well would represent a worst case scenario.

Roadside ditches are typically high organic soils with grasses similar to wetland soils. In fact, frequently inundated roadside environments may meet wetland criteria classification as evidenced by the dominance of water-loving vegetation such as cattails. With the exception of very large spills, these typical conditions would cause spills to run off and affect only the uppermost layers of soil. Dry roadside soils, on the other hand, permit the infiltration of spilled and applied herbicides. Therefore, the antecedent weather and ground water conditions at the time of a spill are an important factor.

Ironically, the presence of roadside groundcover (weeds) affects the fate of spilled herbicides. The very same weeds we try to destroy may offer a biologically active buffer zone to limit the extent of roadside spills. The interactions between roadside vegetation and spilled chemicals is poorly understood.

Other factors determining the spatial extent of a spill are the physical state of the chemical and its chemical properties. The hydrophilic chemical studied in this report, 2,4-D, is often transported in a dry form. The leaching and runoff of dry chemicals will depend on their solubility and the amount of water present in the roadside environment.

Long term costs of roadside spills will not only depend on the characteristics of individual spills, but also on the number of spills per year. Long term data on the frequency and type of spills are important for deciding on the potential return on capital investments. The high costs of cleaning up spills underscores the economic importance of preventing spills.
7.3 Fate of Roadside Herbicides

Once a herbicide is spilled or applied to a soil environment it may: 1) absorb to the soil, 2) leach to groundwater or 3) dissolve in runoff water. In fact, much less than 1 percent of an applied herbicide accounts for the herbicidal effect (176). The remaining 99 percent is ineffective. Herbicides sorbed on soil particles may end up in runoff with the eroded soil. Therefore, the initial fate of a herbicide is associated with either the soil or the soil water.

There are several complex modeling approaches to estimate the leaching and runoff potential of herbicides in a given soil environment. For practical purposes, simpler models based on a few simple factors, can predict the general fate of herbicides. The leaching potential of a herbicide (176) is best predicted by its: water solubility (> 30 ppm), partition coefficient, $K_d$ (<5), hydrolysis half life (> 25 weeks), and soil half life (> 2 weeks). If a herbicide meets any one of these criteria, it has a potential to leach. The mobility of a herbicide is controlled largely by its adsorption characteristics and its hydrolysis reactions. Rainfall is also a major factor influencing pesticide movement. Heavy rain following an application or spill is likely to move the pesticide deeper into the soil profile (177).

Mobilities of various herbicides in soils are listed in Table 24. Class 5 compounds are very mobile and Class 1 are relatively immobile. The herbicide used in this study, 2,4-D, a Class 4 pesticide, is likely to leach rather than sorb on the soil.

7.4 Comparison of Different Cleanup Method Costs

The human health risks of herbicides in water are difficult to quantify. For example, the acute toxicity in terms of mg/kg of body weight of table salt is greater than the simazine herbicides (177). Based on Lost Life Expectancy analysis (178), herbicides pose very little health risks compared to other factors, such as smoking or poverty. Moreover, detrimental health risks of herbicides are based on the toxicity of the pesticide multiplied by long-term exposure (drinking 2 liters of water everyday for several decades). In the case of roadside spills, exposures are rare and of short duration. Hence, it is difficult to assign dollar values to health risks that result from roadside spills of herbicides. A qualitative analysis of environmental risks for agricultural applications of herbicides (179) is presented in Figure 48. Environmental damages can be represented with a relative scale of 0 (no damage) to 100.
## Table 24

**Relative Mobility of Herbicides in Soils** (Close and Canter, 1990)

<table>
<thead>
<tr>
<th>Mobility Class</th>
<th>Class 5</th>
<th>Class 4</th>
<th>Class 3</th>
<th>Class 2</th>
<th>Class 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Mobile</strong></td>
<td>TCA</td>
<td>Picloram</td>
<td>Propachlor</td>
<td>Siduron</td>
<td>Neburon</td>
</tr>
<tr>
<td></td>
<td>Dalapon</td>
<td>Fenac</td>
<td>Denuron</td>
<td>Bensulide</td>
<td>Chloroxuron</td>
</tr>
<tr>
<td></td>
<td>2,3,6-TBA</td>
<td>Pyrichlor</td>
<td>Prometone</td>
<td>Prometryne</td>
<td>DCPA</td>
</tr>
<tr>
<td></td>
<td>Tricamba</td>
<td>MCPA</td>
<td>Naptalam</td>
<td>Terbutryn</td>
<td>Lindane</td>
</tr>
<tr>
<td></td>
<td>Dicamba</td>
<td>Amitrole</td>
<td>2,4,5-T</td>
<td>Propanil</td>
<td>Phorate</td>
</tr>
<tr>
<td></td>
<td>Chloramben</td>
<td>2,4-D</td>
<td>Terbacil</td>
<td>Diuron</td>
<td>Parathion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dinoseb</td>
<td>Propham</td>
<td>Linuron</td>
<td>Disulfoton</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bromacil</td>
<td>Fluometuron</td>
<td>Pyrazon</td>
<td>Diquat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norea</td>
<td>Molinate</td>
<td>Clorphenamidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diphenamid</td>
<td>EPTC</td>
<td>Dichlormate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thionazine</td>
<td>Chlorthiamid</td>
<td>Ethion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Endothall</td>
<td>Dichlobenil</td>
<td>Zineb</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Monuron</td>
<td>Vernolate</td>
<td>Nitratin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Atratone</td>
<td>Pebulate</td>
<td>C-6989</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WL 19805</td>
<td>Chlorpropham</td>
<td>ACNQ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Atrazine</td>
<td>Azinphosmethyl</td>
<td>Morestan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Simazine</td>
<td>Diazinon</td>
<td>Isodrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ipazin</td>
<td>Benomyl</td>
<td>Dieldrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alachlor</td>
<td>Chloroneb</td>
<td>Paraquat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ametryne</td>
<td>Trifluralin</td>
<td>Benefin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Propazine</td>
<td>Heptachlor</td>
<td>Benendrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trietazine</td>
<td>Endrin</td>
<td>Aldrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chlorodane</td>
<td>Toxaphene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DDT</td>
</tr>
</tbody>
</table>

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Figure 48  Cost-environmental hazard index frontier for selecting cost-effective strategies to reduce environmental hazard of pesticide use.
A similar approach can be applied to roadside spills. For a given set of spill conditions, different treatment strategies yield different points on the graph. The group of least cost treatment methods for a given level of risk reduction yields a frontier curve. Prior to a spill, we may assume that the health risks of the roadside environment are not dangerous. During and immediately following a spill, the health risks will reach a maximum. These risks will diminish at a rate dependent on the selected treatment methods (Figure 49).

7.5 Cost Comparison of Biodegradation with Other Treatments

The high costs of landfilling and incineration make bioremediation techniques a cost effective option when they are technically feasible (i.e., when the spilled chemical is a biodegradable organic compound). Table 25A and Table 25B compare the costs and benefits of bioremediation and landfilling based on the cost elements that make up a remediation effort. Assuming that bioremediation is the best alternative, the economic question reduces to whether or not the benefits of providing acclimated microorganisms would outweigh their cost compared to the costs and benefits of using natural soil microorganisms.

7.6 Cost Comparison of Specially Seeded Biodegradation with Natural Biodegradation

Bioremediation options consist of three potential type of bacterial seeds: 1) specialty (acclimated) bacteria, 2) activated sludge (taken from aeration tanks of domestic or industrial wastewater plants, or 3) naturally occurring soil bacteria. Natural microbes are free compared to activated sludge (0 - 5,000 dollars) and specialty bacteria (1,000 - 100,000 dollars). However, the use of natural soil bacteria is not always technically feasible. Acclimated bacteria become economically feasible when: 1) time requirements are an important constraint or 2) the natural soil microorganisms have been destroyed by the spilled chemical. The short duration of accelerated biodegradation minimizes operation costs (labor), minimizes the duration of traffic interference, and limits the spatial extent of pollution (Table 26).
Figure 49 Theoretical biodegradation rates for two different bacterial seeds, expressed in terms of the Environmental Hazard Index
TABLE 25A
Qualitative Comparison of Bioremediation and Landfill Disposal
Costs and Benefits

<table>
<thead>
<tr>
<th>Costs</th>
<th>Bioremediation</th>
<th>Landfill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excavation</td>
<td>optional</td>
<td>yes</td>
</tr>
<tr>
<td>Trucking</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Slurry Equipment</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Bacterial Seed, Nutrients</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Replacement Soil</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

Benefits

<table>
<thead>
<tr>
<th>Benefits</th>
<th>Bioremediation</th>
<th>Landfill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in Health Hazard</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Fate of Pollutant</td>
<td>eliminated</td>
<td>transferred</td>
</tr>
<tr>
<td>Applicable for Toxic Spills</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>(heavy metals)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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TABLE 25B
Quantitative Cost Comparison of Bioremediation and Landfill Disposal
of Toxic and Hazardous Materials

<table>
<thead>
<tr>
<th></th>
<th>Bioremediation</th>
<th>Landfill</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capital Investments</strong></td>
<td>(1993 dollars)</td>
<td>(1993 dollars)</td>
</tr>
<tr>
<td>Land</td>
<td>0</td>
<td>10,000 - 100,000 per acre</td>
</tr>
<tr>
<td>Slurry Treatment System</td>
<td>10,000 - 100,000</td>
<td>0</td>
</tr>
<tr>
<td>Bacterial Seed</td>
<td>10,000 - 100,000/herbicide</td>
<td>0</td>
</tr>
<tr>
<td>Excavation</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Trucking</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Labor</td>
<td>30/hour</td>
<td>30/hour</td>
</tr>
<tr>
<td>Replacement Soil</td>
<td>0</td>
<td>10 - 100</td>
</tr>
<tr>
<td>Landfilling</td>
<td>0</td>
<td>100 - 1,000</td>
</tr>
<tr>
<td>Costs</td>
<td>Natural Bioremediation</td>
<td>Seeded Bioremediation</td>
</tr>
<tr>
<td>----------------------------</td>
<td>------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Excavation</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Duration of Labor</td>
<td>&gt; 2 weeks</td>
<td>1 - 2 days</td>
</tr>
<tr>
<td>Slurry Equipment</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Bacterial Seed</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Nutrients and Oxygen</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Benefits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction in Health Hazard</td>
<td>slow</td>
<td>fast</td>
</tr>
<tr>
<td>Potential Spatial Extent of Spill due to Leaching</td>
<td>large</td>
<td>small</td>
</tr>
<tr>
<td>Traffic Interference</td>
<td>&gt; 2 weeks</td>
<td>1 - 2 days</td>
</tr>
<tr>
<td>Applicable for Highly Concentrated Spills</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>
7.7 Development of Cost Functions

Cost functions are developed in order to decide between natural and seeded bioremediation for a soil slurry type system. The essential economic factors are found to be 1) the cost of the seed, \( C_s \); 2) the required time of treatment, \( t \); 3) operational costs, \( C_{op} \); and 4) secondary costs, \( C_2 \). The following cost functions are based on the assumption that the slurry method is identical for both natural and seeded biodegradation. No additional equipment or labor are required for seeded treatment. Hence, both the capital and operational costs for natural and seeded bioremediation are approximately equal. It is assumed that the natural soil microorganisms are not destroyed by the spill.

Chapter 6 of this report demonstrated the technical feasibility of using a slurry type system with acclimated bacteria to provide rapid treatment times (< 24 hours) of high concentrations of 2,4-D in soil water. Slurry systems are well suited for contaminated soil that has poor hydraulic characteristics. A slurry type system relies on mixing the soil in situ with the acclimated bacteria. Mixing is provided by a number of augers, which move the wet soil both upward and downward. Slurry systems work well for shallow spills of organic compounds.

7.8 Cost Functions

The total cost of cleanup, \( C_T \), for natural bioremediation (no seed, only nutrients and oxygen) is

\[
C_T = C_C + C_{op} t_N + C_2 t_N
\]

(90)

where \( t_N \) is the time of cleanup for natural biodegradation and

\( C_C \) is the capital cost.

The total cost of cleanup, \( C_T \), for seeded bioremediation (seed, nutrients, and oxygen) is

\[
C_T = C_C + C_{op} t_s + C_2 t_s + C_s
\]

(91)

where \( t_s \) is the time of cleanup for seeded biodegradation.

To estimate the cost difference between natural and seeded biodegradation we subtract Equation (91) from Equation (90) to obtain

\[
\Delta C_T = (C_{op} + C_2) (\Delta t) - C_s
\]

(92)

where \( \Delta C_T \) is the total cost difference between natural and seeded bioremediation and

\[
\Delta t = t_N - t_s.
\]

(93)
\( \Delta t \) can be thought of as the reduction in cleanup time caused by using an acclimated seed instead of the naturally occurring soil microorganisms. The actual value of \( \Delta t \) depends on many site specific factors; a typical value is 2 weeks. If \( \Delta C_T \) is positive, then using a seed is the least cost approach. If \( \Delta C_T \) is negative, then using a natural soil bacteria is the least costly approach. Setting \( \Delta C_T \) equal to zero in Equation (92) defines the cost of a seed, which makes the costs of unseeded and seeded approaches equal. For \( \Delta C_T = 0 \), the cost of the seed is given by

\[
C_s = \Delta t \left( C_{op} + C_2 \right)
\]

(94)

or

\[
C_s = \Delta t \left( C_D \right)
\]

(95)

where \( C_D \) is the daily cleanup cost.

If the cost of the seed is greater than \( C_s \) calculated using Equation (94), then natural bioremediation is the most economical choice.

An alternative way to decide between natural and seeded biodegradation is presented in Figure 50. Again, the critical parameters are the daily costs of treatment (including secondary costs), the cost of the specialty bacteria, and the acclimation time of the natural soil bacteria \((t_N - t_a)\). Plotting Equation (92) in the form shown in Figure 50 permits one to select the least cost alternative based on the daily costs. The point where the line intersects the horizontal axis is the point where the cost of seeded biodegradation is equal to natural biodegradation. Specialty bacteria are the least costly alternative when daily costs are high (to the right of the intersection point). Natural biodegradation is the least costly option when daily costs are low (to the left of the intersection point). These daily costs are directly related to secondary costs, such as the cost of traffic interference or shutting down a public water supply. If the daily costs of treatment are greater than \( C_s/\Delta t \), then using acclimated bacteria is the least costly option.

7.9 Example: Comparison of Natural and Accelerated Biodegradation of a Roadside Spill near a Public Water Supply Well - Cost Functions

Problem Statement: Several hundred gallons of 2,4-D are spilled along a roadside near a shallow well. The spill contaminates the top 0.5 m. of soil along 20 m. of ditch. The nearby well is shut off, at a cost of $500 per day, so no 2,4-D will be drawn toward the well. An in-situ soil slurry, bioremediation method is selected as the most technically feasible option. Daily
Figure 50  Least Cost Regions as a Function of Daily Costs
operational costs of cleanup are $1520 per day. The acclimation time of the natural bacteria is 12 days. Should natural or specialty bacteria be utilized?

Solution: Using the cost function of Equation (94):

\[ C_s = \Delta t \left( C_{op} + C_f \right) = 12 \left( 1520 + 500 \right) = \$ 18,240 \]

Therefore, if the cost of the seed is greater than $18,240, then natural bioremediation is the least costly alternative.

7.10 When should acclimated bacteria be used instead of naturally occurring bacteria?

If bioremediation of a roadside spill is the best alternative compared to landfilling or incineration, the economic question reduces to whether or not to use acclimated bacteria instead of naturally occurring bacteria. This question can be answered by comparing the costs and benefits of unseeded versus seeded treatment methods. Accelerated bioremediation using bacterial seeds is economically feasible for small spills affecting the topsoil and requiring quick remediation. For example, spills which interfere with traffic conditions are well suited for accelerated biodegradation. Using acclimated bacteria reduces the treatment time and therefore, minimizes the costs of both traffic problems and treatment operation. Acclimated bacteria are economically attractive when daily costs of cleanup are high, as is the case when a public water supply must be shut off. Specialty bacteria should also be used when the natural soil microorganisms have been destroyed by the spilled herbicide.

7.11 Decision Aids for Management of Herbicide Spills

Based on the economic analysis, accelerated bioremediation of a roadside spill is preferred to natural bioremediation when:

1. The natural bacteria are destroyed by the spilled chemical.
2. The length of time required for natural bioremediation would result in undesirable migration of the pollutant (affecting a public water supply).
3. The length of time required for natural bioremediation would result in undesirable traffic problems.
4. Public pressures demand prompt and effective clean-up.
7.12 Management Strategies to Minimize Pollution from Roadside Herbicides

The unique hydrology of Louisiana, characterized by high water tables and the dominance of wetlands, affects the fate of roadside chemicals. During the spring, many roadside soils are submerged, resulting in anaerobic conditions which slow the degradation of herbicides. A planning index is presented in this section to assess potential pollution in Louisiana from roadside chemicals. The index incorporates method, timing, and rate of herbicide application, soil properties, ground water and surface water conditions, and herbicide properties. If applied, the index would identify the most environmentally vulnerable regions of the state. Identified areas are those most in need of monitoring. With limited financial resources, a worst case monitoring effort is very economical and practical. The index also helps to select the proper herbicide for a given roadside in order to minimize environmental risks. For example, in porous soils with high groundwater tables (near the surface) hydrophobic herbicides should be used to minimize leaching.

Indices are used in agriculture to assess the potential of agricultural chemicals to leach to groundwater (176, 177, 179). The higher the calculated value of the index (for a given herbicide applied to a given field situation) the greater the risk of off-site pollution. For example, a water soluble herbicide applied to a sandy soil with a high water table would produce a very high index value.

A similar scheme can be used to assess the potential environmental impacts of roadside herbicides. Pollution index values give a relative weight (typically an integer value from 0 to 5) to the factors which determine the fate of an applied herbicide. Factors can be given equal weight or varied according to their relative importance. The most significant factors have a weight of 5. Typical factors include properties of the soil and herbicide. More complicated indices might include population density, public water supply locations, and herbicide toxicity. Controllable factors are: selection of the herbicide, method of application (sprayed in solution or injected dry), application rates, and timing of application (during optimal weather conditions). It is the task of managers to minimize pollution risks by selecting the optimal combination of controllable factors, at a minimum cost. Indexing herbicides and soil conditions permits managers to compare the environmental risks of two different herbicides or to rank the effectiveness of numerous management strategies. Moreover, indices enable identification of
the most vulnerable regions of the state, where careful application and field monitoring are most important.

Indices may be divided into a herbicide index, based on properties of the herbicide, and a groundwater pollution index, based on the hydrogeologic setting. Hence, the value of the pollution index will be the product of the herbicide index and the groundwater pollution index. A simple herbicide index (HI) which gives equal weight to application rate, mobility, and degradation is given by (177)

\[
HI = UMD
\]  \hspace{1cm} (96)

where
\begin{align*}
U & = \text{usage, application rate scaled from 0 to 5 (see Table 27)} \\
M & = \text{mobility class scaled from 1 to 5 (see Table 24)} \\
D & = \text{degradation half life scaled from 1 to 5; higher number indicates longer degradation half life (Table 27).}
\end{align*}

The above herbicide index enables the comparison of the environmental risks of different herbicides. It can be combined with a groundwater pollution index to account for hydrogeologic factors of a given area. DRASTIC (176) is one simple groundwater pollution index to evaluate the likelihood of a herbicide reaching the groundwater table. Table 28 shows the hydrogeologic factors used to calculate the pollution potential in a given physical setting. Depth to water table has the highest weighting (5) of all factors. DRASTIC index values range from 53 to 224 for all hydrogeologic settings in the U. S. (176). By multiplying the herbicide index and the groundwater pollution index, the pollution potential for a given herbicide in a given area can be assessed.

An alternative to using DRASTIC or other simple leaching models is to use an aquifer recharge potential map based on soil and aquifer properties. Soil Survey Maps from the Soil Conservation Service provide valuable information on the leaching and runoff potential at any given location in Louisiana. On a larger scale, the Louisiana Department of Environmental Quality publishes Aquifer Recharge Maps showing the vulnerability of aquifers to groundwater contamination. The maps provide a quick visual aid to identify areas susceptible to pollution.

Indices are useful because they enable managers of roadside chemicals to evaluate potential environmental risks of different herbicides and locations. Once the risks of applying a certain herbicide on a specific region are ranked, planners may quickly identify areas most in
TABLE 27  
WEIGHTING FACTORS FOR HERBICIDE INDEX (177)

<table>
<thead>
<tr>
<th>Weighting Factor</th>
<th>(lb/acre) Usage, U</th>
<th>(Weeks) Degradation Half Life, D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 0.01</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>0.01 - 0.2</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>2</td>
<td>0.2 - 0.5</td>
<td>1 - 3</td>
</tr>
<tr>
<td>3</td>
<td>0.5 - 1.0</td>
<td>3 - 6</td>
</tr>
<tr>
<td>4</td>
<td>1.0 - 2.0</td>
<td>6 - 12</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>&gt; 12</td>
</tr>
<tr>
<td>Feature</td>
<td>Weighting Factor</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Depth to Water Table</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Net Recharge</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Aquifer Media</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Soil Media</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Topography</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Impact of Vadose Zone</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Hydraulic Conductivity of Aquifer</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
need of monitoring for herbicide pollution. This worst case approach is economical, because the field data collection requirements are minimized. If no herbicide contamination is found in the most vulnerable regions, then the likelihood of problems in other areas is small. Current management efforts should focus on the regions of the state with the highest environmental risks, as identified by pollution indices and input from field personnel. Statewide monitoring efforts which do not include susceptible areas tend to underestimate the impact of roadside chemicals on local environments.

Based on the above discussion, planners and managers can use the information that goes into pollution indices to select optimal management strategies. The environmental objective is to select the controllable factors (application timing, rate, method...) given the fixed factors (soil type, depth to groundwater, drainage to wetlands...) of a given roadside. The following strategies are presented as an example of this approach.

**Strategies for the Minimization of Pollution from Roadside Chemicals**

1. Match herbicide to soil conditions (i.e., use a hydrophobic chemical for applications on porous soils).
2. Apply herbicide during dry weather.
3. Develop alternative roadside vegetation and weed control practices (i.e., lime, alternative vegetation...).
4. Consider enhancing biodegradation of herbicide with specialty bacteria.
5. Control runoff with simple filter beds such as bales of straw.
6. Give special attention to herbicide applications near anaerobic bodies of water (i.e., wetlands and bayous).

**7.13 Analysis Based on Costs of Alternative Remediation Methods**

Another approach to cost analysis is to use cost figures that have been developed by experienced workers in the remediation field. This approach results in cost ranges for each remediation alternative. The costs are expressed in units including dollars per cubic yard of soil or dollars per ton of soil. The cost figures developed by experienced workers have an advantage of simplicity in that the user does not have to specify the details of the equipment, supplies, and labor to be used in the remediation activity. However, the costs are expressed in terms of
ranges so the cost figures are not suitable to discriminate between two alternatives which are in the feasible range of each remediation alternative. However, taking the advantages and disadvantages of this method of estimating costs into account leads to the conclusion that it provides a rapid method for preliminary cost estimates.

Table 29 shows a tabulation of the costs of alternative treatment and disposal alternatives (Mark Zappi, personal communication, November 10, 1993). The costs are presented in terms of dollars per ton of soil. They would be nearly identical in units of dollars per cubic yard of soil. The costs vary considerably for one technology. For example, incineration ranges from $350 per ton to $1,200 per ton. Part of this variation is a scale effect. If one is incinerating only one ton of material, the unit cost will be high, (approximately $1,200 per ton), while if one is incinerating a large volume of readily combustible material that is lightly contaminated by hazardous material the cost may be as low as $350 per ton. Another factor that has an impact on the cost range is the degree of hazard of the contaminant. A higher hazard will result in a higher unit cost. A herbicide is expected to be in the middle hazard range. Application of the cost figures will be explained through examples.
TABLE 29
Costs of Bioremediation, Landfilling, Incineration, Solidification and Stabilization, and Low Temperature Thermal Desorption

<table>
<thead>
<tr>
<th>Treatment Method</th>
<th>Treatment Cost (1993 Dollars / Ton of Soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incineration</td>
<td>350 - 1,200</td>
</tr>
<tr>
<td>Secure Landfilling</td>
<td>100 - 1,000</td>
</tr>
<tr>
<td>Solidification Stabilization and Landfilling</td>
<td>80 - 300</td>
</tr>
<tr>
<td>Low Temperature Thermal Desorption</td>
<td>200 - 500</td>
</tr>
<tr>
<td>In Situ Bioremediation</td>
<td>40 - 120</td>
</tr>
</tbody>
</table>
Example 1
A liquid herbicide was spilled on a sandy soil. Remediation efforts were undertaken immediately after the spill. It is estimated that 100 cubic yards of soil are contaminated. What are estimates of the remediation costs?
Here one has a concentrated spill that has contaminated the surface and near surface soil. Removal of the soil is an option that is recommended so that the herbicide can be transported away from the site before it spreads and contaminates a larger soil volume. Table 29 suggests that the treatment and disposal options to consider are: incineration; secure landfilling; or solidification, stabilization and landfilling. One would rule out low temperature thermal desorption of the herbicide as being technically unfeasible due to the unavailability of the proper equipment at the site in a short time. In situ bioremediation may be unfeasible due to the need to clean up the site quickly. The choice between incineration, secure landfilling, and solidification, stabilization and landfilling would rest on the costs. Table 29 suggests incineration would be more costly than the other two alternatives as its most favorable cost is higher than the lowest cost for secure landfilling or solidification, stabilization and landfilling: $350/ton versus $100 to $1,000/ton or $350/ton versus $80 to $300/ton. Bids on the feasible treatment and disposal alternatives could be used to settle which alternative to employ.

Example 2
A roadside ditch has become contaminated with a low level concentration of herbicide due to too frequent application with a too concentrated solution of herbicide. Exploration suggests the herbicide is in the upper 6 in. of soil. The contaminated region is about 20 ft. wide and 1,800 ft. long. Some low concentration of herbicide has spread deeper into the soil, approximately 18 inches. There is not an urgent need for remediation, but neither can the problem be ignored. This situation favors in situ bioremediation. There is widespread contamination. Time is available for bioremediation. The contamination is in the near surface region. There are regions of low level contamination that may be remediated by bacteria. If acclimated bacteria are used, one can cultivate them into the topsoil with conventional farm equipment. The encouragement of remediation using natural bacteria could be tried if sufficient time is available. Nutrients could be cultivated into the soil to encourage the growth of natural bacteria.
This site's remediation cost would be projected at the lower range of in situ bioremediation, say ($50/ton) x (1 ton/cu. yd.) x (6/12 ft.) x (20 ft.) x (1800 ft.) x (1 cu. yd. / 27 cu. ft.) = $22,222.

7.13 Summary

Accelerated bioremediation using bacterial seeds is economically feasible for small spills affecting the topsoil and requiring quick remediation. Specialty bacteria should also be used to clean up organic spills when the natural soil microorganisms have been destroyed by the spilled herbicide. A soil slurry treatment system is recommended in order to uniformly distribute oxygen and bacteria to all of the contaminated soil. For deeper spills, groundwater should be pumped and treated with acclimated bacteria in an activated sludge process. Successful injection of acclimated bacteria to polluted regions of an aquifer is not technically reliable at this time. The main problem is based on the inability to supply oxygen and bacteria to the impermeable regions of the aquifer.

Costs of bioremediation are generally equal or less than alternative treatment methods such as landfilling or incineration. Assuming that bioremediation is the selected treatment method, the question becomes whether or not to supply acclimated bacteria. Specialty bacteria are well suited for spills which incur high daily costs of treatment, as is the case when the spill interferes with traffic or a public water supply. In order to compare the costs of natural and seeded bioremediation, three parameters must be quantified. The essential economic factors are: 1) the difference in treatment time between natural biodegradation and seeded biodegradation, Δt, 2) the cost of supplying the seed, C_S, and the daily costs of treatment, C_D. If the daily costs, C_D, of treatment are greater than C_S/Δt, then using acclimated bacteria is less costly than using natural bacteria.
8. CONCLUSIONS

In situ remediation requires the knowledge of processes associated with the contaminant transport. In natural soils the contaminant may move preferentially through large voids causing increased groundwater pollution potential. In this study 2,4-D transport was more influenced by the physical properties rather than the chemical sorption behavior. For a Bt horizon of a silt loam, the transport of 2,4-D in columns with aggregates was successfully described by the Two-Site/Two-Region Model assuming linear sorption. Even though the sorption isotherm was nonlinear, the linearized modelling approach was sufficient to describe the data. However, when the solution was passed through the soil matrix, the convective dispersive equation was not successful to describe the data. Furthermore, the retardation factors determined under batch experiments were not accurate for predicting transport. Hence, when roadside soils are investigated for assessing herbicide movement, column experiments should be used to determine pollution potential. Furthermore, the in situ transport regime cannot be assessed a priori. In fact, field tests need to be carried out to determine whether or not rapid bypass flow will contribute to groundwater contamination. Both the column and two-dimensional flume studies revealed that bypass flow may be the most important factor in subsurface pollution.

In order to assess the potential for bacteria to degrade herbicides in soil environments, one needs to analyze the behavior of bacteria in soil systems. The model developed in this study can be used to determine the potential for adding bacteria to contaminated soils. The experimental bacteria transport study revealed that clogging may be an important factor when the suspension is introduced into a homogeneous soil matrix. Hence, preferential flow patterns may be equally important for bacteria transport as herbicide movement.

The biodegradation study revealed that batch reactors are far more efficient in the degrading of organic pollutant than column methods. Overall, the microorganisms were able to degrade 2,4-D. This was also confirmed by the biodegradation data of the soil flume.

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Accelerated bioremediation using bacterial seeds is economically feasible for small spills affecting the topsoil and requiring quick remediation. Specialty bacteria should also be used to clean up organic spills when the natural soil microorganisms have been destroyed by the spilled herbicide. A soil slurry treatment system is recommended in order to uniformly distribute oxygen and bacteria to all of the contaminated soil. For deeper spills, groundwater should be pumped and treated with acclimated bacteria in an activated sludge process. Successful injection of acclimated bacteria to polluted regions of an aquifer is not technically reliable at this time. The main problem is based on the inability to supply oxygen and bacteria to the impermeable regions of the aquifer.

Costs of bioremediation are generally equal to or less than alternative treatment methods such as landfilling or incineration. Assuming that bioremediation is the selected treatment method, the question becomes whether or not to supply acclimated bacteria. Specialty bacteria are well suited for spills which incur high daily costs of treatment, as is the case when the spill interferes with traffic or a public water supply. In order to compare the costs of natural and seeded bioremediation, three parameters must be quantified. The essential economic factors are: 1) the difference in treatment time between natural biodegradation and seeded biodegradation, $\Delta t$, 2) the cost of supplying the seed, $C_s$, and 3) the daily costs of treatment, $C_d$. If the daily costs, $C_d$, of treatment are greater than $C_s/\Delta t$, then using acclimated bacteria is less costly than using natural bacteria.
9. RECOMMENDATIONS

In order to choose the best cleanup method, the following steps should be taken:

(i) determine the transport regime (convective-dispersive vs bypass flow)
(ii) determine the chemical reactivity in order to assess the retardation of the herbicide
(iii) determine the history, nature, and extent of contamination

Once the properties of the soil and the contamination are known, remedial measures can be taken. In particular, small spills may be efficiently treated by excavating the soil and using a batch bio-reactor to remove the contaminant. A soil slurry treatment system is also recommended in order to uniformly distribute oxygen and bacteria to all of the contaminated soil. For deeper spills, groundwater should be pumped and treated with acclimated bacteria in an activated sludge process.

Based on the economic analysis, accelerated bioremediation of a roadside spill is preferred to natural bioremediation when:

1. The natural bacteria are destroyed by the spilled chemical.
2. The length of time required for natural bioremediation would result in undesirable migration of the pollutant (affecting a public water supply).
3. The length of time required for natural bioremediation would result in undesirable traffic problems.
4. Public pressures demand prompt and effective clean-up.

The following strategies are recommended to minimize pollution from roadside chemicals:

1. Match herbicide to soil conditions (i.e., use a hydrophobic chemical for applications on porous soils).
2. Apply herbicide during dry weather.
3. Develop alternative roadside vegetation and weed control practices (i.e., lime, alternative vegetation...).
4. Consider enhancing biodegradation of herbicide with specialty bacteria.
5. Control runoff with simple filter beds such as bales of straw.
6. Give special attention to herbicide applications near anaerobic bodies of water (i.e., wetlands and bayous).

The unique hydrology of Louisiana, characterized by high water tables and the dominance of wetlands, affects the fate of herbicides. Field monitoring of the impact of
roadside chemicals should be conducted on the most vulnerable regions of Louisiana. These areas can be identified using the pollution index presented in Chapter 7. Areas with the highest value of the pollution index are those most in need of careful application and monitoring. With limited financial resources, a worst case monitoring effort is very economical and practical. The index also helps to select the proper herbicide for a given roadside in order to minimize environmental risks.

In order to compare the costs of natural and seeded bioremediation, three parameters should be quantified. The essential economic factors are: 1) the difference in treatment time between natural biodegradation and seeded biodegradation, $\Delta t$, 2) the cost of supplying the seed, $C_s$, and 3) the daily costs of treatment, $C_D$. If the daily costs, $C_D$, of treatment are greater than $C_s/\Delta t$, then using acclimated bacteria is less costly than using natural bacteria.
10. REFERENCES


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