Review Paper

Engineering scale-up of in situ bioremediation processes: a review

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Received 15 April 1994; accepted after revision 2 March 1995

Abstract

To be useful to field practitioners, advances in bioremediation research must be capable of being scaled up from the laboratory to the field. The phenomena which control the rate at which biodegradation proceeds are typically scale-dependent in nature. Failure to understand and account for scale-dependent variables, such as mass transport limitations, spatial heterogeneities and the presence of competing microorganisms, may inhibit the effectiveness of field-scale bioremediation designs. This paper reviews and evaluates the methods available for characterization of the processes effecting bioremediation at scales ranging from the laboratory to the field. Questions facing the field-scale practitioner of bioremediation are addressed in a manner which highlights the current state of research, the reliability of results and the extent to which laboratory-scale research accurately reflects common field conditions. Where gaps or inadequacies exist in our current knowledge or methods, research needs are identified. This review is intended to complement existing work by providing a framework from which to assess the importance of scale of observation to a particular result or conclusion, thereby providing an integrated approach to the scale-up process.

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SSDI 0169-7722(95)00017-8
1. Introduction

Bioremediation, the engineered application of microbiological processes to clean up soil or groundwater contaminated with chemicals, has emerged as a viable remedial approach for dealing with these environmental problems. Bioremediation is attractive because it has the potential to: (1) permanently eliminate contaminants through biochemical transformation or mineralization; (2) avoid harsh chemical and physical treatments; (3) operate in situ; and (4) be cost effective. While advances have been made in understanding many of the basic phenomena underlying bioremediation, such as elucidation of biochemical degradation pathways and identification of indigenous bacteria capable of biodegrading pollutants, it remains difficult to engineer field-scale bioremediation applications.

Bioremediation involves complex interactions of biological, chemical and physical processes, and requires integration of phenomena operating at scales ranging from that of the microbial cell \(10^{-6} \text{ m}\) to that of the geological site \((10 - 1000 \text{ m})\). By necessity, laboratory investigations of biodegradation are performed at a small scale. Intermediate-scale experiments, with soil columns for example, are frequently used as a bridge between microcosms and a pilot- or field-scale system. Intermediate-scale systems retain much of the control possible in bench-scale experiments, but allow observation of mass transport and interfacial transfer effects on biodegradation rates and contaminant movement. Unfortunately, the extension from a laboratory- or intermediate-scale experimental system to field-scale bioremediation designs often introduces: (1) additional mass transport mechanisms and limitations; (2) the presence of multiple phases, contaminants and competing microorganisms; (3) spatial heterogeneities; and (4) one or more factors which may inhibit bacterial growth, such as unfavorable pH, nutrient, or redox conditions. Thus, what appears to be a viable bioremediation strategy in the laboratory may be unsuccessful in the field (Goldstein et al., 1985; Lee et al., 1988). On the other hand, studies conducted only at the field scale are often hampered by the inability to conclusively prove that contaminant loss is a result of biodegradation and not some abiotic process (Brown et al., 1985). There is a need to better understand which information translates across scales and which does not.

Several review articles have been published which address the physical, chemical and biological conditions necessary to facilitate contaminant biodegradation. The influence of site and contaminant physical interactions such as mass transport (Keely et al., 1986) and sorption (Pignatello, 1989) have received attention. The requirements for optimal microbial growth under a variety of circumstances has been extensively reviewed (Leahy and Colwell, 1990), and degradation pathways have been described in detail for petroleum hydrocarbons (Gibson and Subramanian, 1984). Reviews of field methods available for bioremediation and biotreatability screening of contaminated sites (J.T. Wilson and Ward, 1987; Morgan and Watkinson, 1989; Sims et al., 1990; Hoeppel et al., 1991) have concluded that consideration of scale-dependent phenomena such as mass transport and interfacial transfer mechanisms is prerequisite to success in the field. Rittmann et al. (1992) concluded that the major research needs to facilitate bioremediation success in the field are means to quantify microbial kinetics, sorption kinetics, biologically induced clogging and colloidal transport — all of which are scale-dependent.
The purpose of this article is to review the information and methods available for characterizing constituent processes contributing to bioremediation at different scales and to critically evaluate the utility of these tools for scaling up to a field application. Where gaps or inadequacies exist in current knowledge or methods, research needs are identified. This review is intended to complement existing work by providing a framework from which to assess the importance of scale of observation to a particular result or conclusion, thereby providing an integrated approach to the scale-up process.

2. Phenomena and scales

2.1. Scale definitions

The engineering of bioremediation systems is aided by defining three scales of observation: micro-, meso- and macroscale, as illustrated in Fig. 1. The scale definitions are arbitrary, but serve as a useful conceptual structure for approaching the engineering scale-up problem. The microscale is taken as the scale at which chemical and microbiological species and reactions can be characterized independently of any transport phenomenon. Examples of microscale features are the composition of microbial consortia and the kinetics and stoichiometry of transformation reactions. The physical scale of these phenomena is the dimension of the microbial cell, on the order of $10^{-6} - 10^{-5}$ m. The mesoscale is defined as the scale at which transport phenomena and system
Table 1
Phenomena influencing bioremediation

<table>
<thead>
<tr>
<th>Scale</th>
<th>Representative characterization methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microscale:</strong></td>
<td></td>
</tr>
<tr>
<td>Microorganisms</td>
<td>plate counts, gene probes</td>
</tr>
<tr>
<td>Degradation pathways</td>
<td>batch reaction studies</td>
</tr>
<tr>
<td>Reaction stoichiometry</td>
<td>batch reaction studies</td>
</tr>
<tr>
<td>Reaction kinetics</td>
<td>batch reaction studies</td>
</tr>
<tr>
<td>Electron acceptors</td>
<td>chemical analysis for N, P</td>
</tr>
<tr>
<td>Nutrients</td>
<td>chemical analysis</td>
</tr>
<tr>
<td>Inhibitors, toxicity</td>
<td>batch reaction studies</td>
</tr>
<tr>
<td>Water activity, pH</td>
<td>electrochemical probes</td>
</tr>
<tr>
<td>Reactions with soil or aquifer matrix</td>
<td>abiotic reaction studies</td>
</tr>
<tr>
<td>Chemical equilibria</td>
<td></td>
</tr>
<tr>
<td>Sorption (equilibrium)</td>
<td>abiotic batch sorption studies</td>
</tr>
<tr>
<td><strong>Mesoscale:</strong></td>
<td></td>
</tr>
<tr>
<td>Sorption (non-equilibrium)</td>
<td>abiotic batch and column sorption studies</td>
</tr>
<tr>
<td>Enumeration</td>
<td>biofilm studies, attached microbe</td>
</tr>
<tr>
<td>Diffusion</td>
<td></td>
</tr>
<tr>
<td>Plugging/filtration</td>
<td>column studies, pressure drop and flow rate</td>
</tr>
<tr>
<td>Interphase transport</td>
<td>multiphase column studies</td>
</tr>
<tr>
<td><strong>Macroscale:</strong></td>
<td></td>
</tr>
<tr>
<td>Advection</td>
<td>well elevations, pump tests, tracer studies</td>
</tr>
<tr>
<td>Dispersion</td>
<td>conservative tracer studies</td>
</tr>
<tr>
<td>Spatial heterogeneity</td>
<td>well logs, core permeabilities</td>
</tr>
<tr>
<td>Hydrologic properties and boundary conditions</td>
<td>as for advection and dispersion</td>
</tr>
</tbody>
</table>

Geometry are first apparent, with the exclusion of advective or mixing processes. Mesoscale phenomena include diffusion, sorption and interphase mass transfer. Possible physical scales for mesoscale phenomena include the size of pore channels or soil particles, the characteristic diffusion length, or the dimension of microbial aggregates ($10^{-5}$–$10^{-2}$ m). Advection, dispersion and geologic spatial heterogeneity are examples of macroscale phenomena. The corresponding physical scale for these phenomena ranges from $\sim 10^{-2}$ to $\sim 10^2$ m or even larger. Table 1 summarizes by scale many of the phenomena influencing bioremediation. Phenomena are classified according to the smallest scale at which they can be observed.

2.2. Relationship between scales and engineering bioremediation systems

In situ bioremediation is a complex undertaking which requires an understanding of many physical, chemical and biological phenomena. Observations made at the micro- or mesoscale may not necessarily apply at the field (macro) scale. Observed contaminant loss rates, for example, depend on scale. Table 2 illustrates this point with a compilation
Table 2
Scale dependence of contaminant half-lives

<table>
<thead>
<tr>
<th>Half-life (days)</th>
<th>Ratio of field/laboratory half-lives</th>
<th>Contaminant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>laboratory</td>
<td>field</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>397</td>
<td>aviation gas as TPH</td>
<td>Huling and Bledsoe (1990), Hutchins et al. (1991a)</td>
</tr>
<tr>
<td>3.6</td>
<td>23</td>
<td>gasoline, No. 2 fuel oil as TPH</td>
<td>Block et al. (1989)</td>
</tr>
<tr>
<td>28</td>
<td>111</td>
<td>benzene</td>
<td>Barker et al. (1987)</td>
</tr>
<tr>
<td>6.1</td>
<td>42</td>
<td>toluene (nitrate)</td>
<td>Hutchins et al. (1991a)</td>
</tr>
<tr>
<td>5.6</td>
<td>55</td>
<td>m,p-xylenes (nitrate)</td>
<td>Hutchins et al. (1991a)</td>
</tr>
<tr>
<td>10</td>
<td>73</td>
<td>BTEX</td>
<td>Chiang et al. (1989b)</td>
</tr>
</tbody>
</table>

BTEX = benzene, toluene, ethylbenzene, o-,m-,p-xylenes; TPH = total petroleum hydrocarbons.

of several reports from the scientific literature in which laboratory- and field-scale rates are compared. Field measured half-lives tend to be 4–10 times longer than laboratory-determined values, presumably due to scale-dependent rate limitations. For a given set of environmental conditions there is a single phenomenon which will limit the rate at which bioremediation can proceed. Bioremediation engineering must consider all relevant phenomena in Table 1 to determine which will limit contaminant biotransformation rate for a particular site. Field sites are typically heterogeneous, which can cause different phenomena to limit biotransformation rates across the site. Selection of a remedial strategy should include an assessment of its effects on biotransformation rate-limiting phenomena. This assessment is useful for determining: (1) the potential for successful bioremediation; (2) whether the rate can be enhanced; (3) how to best engineer the process; and (4) how to verify bioremediation has occurred. These issues cannot be properly addressed by observations made at a single scale alone.

The focal point of this review are questions which a field practitioner of bioremediation must answer to determine both the feasibility of designing an in situ biotreatment

Table 3
Important questions at each scale of observation

<table>
<thead>
<tr>
<th>Scale</th>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro</td>
<td>- What is the activity of biodegrading microorganisms at the site?</td>
</tr>
<tr>
<td></td>
<td>- Will biotransformation reduce the concentration of contaminant sufficiently?</td>
</tr>
<tr>
<td></td>
<td>- What chemical species limits the extent of biotransformation?</td>
</tr>
<tr>
<td></td>
<td>- What are the intrinsic kinetics of biotransformation?</td>
</tr>
<tr>
<td>Meso</td>
<td>- Does sorption influence the rate of bioremediation?</td>
</tr>
<tr>
<td></td>
<td>- Does interphase transport influence the rate of bioremediation?</td>
</tr>
<tr>
<td>Macro</td>
<td>- Does advective–dispersive transport limit the rate of biodegradation?</td>
</tr>
<tr>
<td></td>
<td>- How does spatial heterogeneity impact bioremediation?</td>
</tr>
<tr>
<td>Overall</td>
<td>- How can bioremediation rates be evaluated and predicted?</td>
</tr>
<tr>
<td></td>
<td>- How does temperature influence the rate of bioremediation?</td>
</tr>
<tr>
<td></td>
<td>- How can bioremediation be modeled?</td>
</tr>
<tr>
<td>Method</td>
<td>Description</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Heterotrophic plate counts</td>
<td>aqueous suspension is inoculated onto sterile agar either while still liquid (pour plate) or after agar has dried (spread plate)</td>
</tr>
<tr>
<td>Hydrocarbon degrader plate counts</td>
<td>similar to heterotrophic plate counts except specific hydrocarbon (or group of hydrocarbons) is only carbon source for growth</td>
</tr>
<tr>
<td>Epifluorescence direct counts</td>
<td>sample is stained with one of several fluorescent stains; counts are then made directly under the microscope</td>
</tr>
<tr>
<td>Most probable number techniques</td>
<td>samples are incubated with reaction specific indicators; color change, gas or precipitate formation indicates the presence/activity level of target culture; bacterial density is predicted by the observed distribution of positive and negative results</td>
</tr>
<tr>
<td>Oligonucleotide probes</td>
<td>labelled (fluorochrome) DNA or RNA complementary sequences for a target sequence in the organism are added to an environmental sample; observe cells by microscopy for label (e.g., fluorochrome)</td>
</tr>
<tr>
<td>Lipid assays</td>
<td>lipids are extracted from pure culture and analyzed in a gas chromatograph; chromatogram is compared to standard library for species match</td>
</tr>
<tr>
<td>Metabolic indicators</td>
<td>tests utilize the capacity of a specific species to make a measurable color or size change due to the degradation of a specific substrate</td>
</tr>
</tbody>
</table>
solution and the steps necessary for success. Questions are organized according to whether they involve phenomena at the micro-, meso-, macroscale, or some combination of scales (Table 3). After identifying the governing phenomena relevant to each question, existing methods for characterizing and quantifying the phenomena are reviewed and evaluated based on how well information obtained by these methods scales up and the extent to which current literature adequately addresses the importance of each phenomenon to biotransformation.

3. Microscale phenomena

3.1. What is the activity of biodegrading microorganisms at the site?

Successful biodegradation depends on the presence and activity of a microbial population capable of degrading the target contaminant(s). Hydrocarbon degrader numbers have been correlated with prior exposure of an ecosystem to contamination. Atlas (1981) reports that hydrocarbon degraders may constitute < 0.1% of total organisms in pristine systems, but up to 100% of total counts in contaminated systems. However, hydrocarbon contamination does not a priori indicate the presence of a population capable of its degradation, particularly if more easily metabolized substrates are present (Swindoll et al., 1988; Leahy et al., 1990). Methods of bacterial enumeration from soil–water systems include heterotrophic plate counts, epifluorescence direct counts, hydrocarbon-degrader plate counts, most probable number (MPN) techniques, DNA (deoxyribonucleic acid) probes, lipid assays, metabolic indicators and radioisotopic methods (Table 4). Direct counts include both metabolically active and inactive organisms, while heterotrophic plate counts theoretically measure only the fraction capable of growth. MPN techniques and DNA probes indirectly measure microbial density through chemical changes brought about due to metabolite production (MPN) or extraction and quantitation of cell genetic material (DNA probes). Lipid assays quantify the fatty acid content of cell suspensions and relate this to the total microbial population. ATP (adenosine triphosphate) assessment methods use the ratio of ATP and ADP (adenosine diphosphate) to the total pool of available adenylate as a measure of microbial activity. Metabolic indicator methods include tetrazolium dye reduction (INT-formazan) \(^1\)

\(^1\) INT = 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride.
the measurement of the ratio of cell ATP to total adenylate available. Radioisotopic methods measure the kinetics of cell uptake of tritiated or $^{14}$C-labelled compounds (tritiated-thymidine or -acetate) and translate this uptake into cell activity estimates (O'Carroll, 1988).

Several researchers have compared the relative usefulness of one technique over another in qualitative assessment of biodegradation capacity. In isolating hydrocarbon degraders from an environmental sample, Wyndham and Costerton (1981) found that plate count enumerations more accurately reflected in situ microbial activity than direct counts and MPN techniques. Conversely, Atlas (1981) reports better enumerations using MPN procedures over plate counts. Researchers have also used the ratio of total organisms to hydrocarbon degraders as a measure of fitness to degrade hydrocarbon contamination. Because of their selectiveness for single species, DNA probes are useful mainly in the laboratory or in a field situation where the fate of an individual organism is tracked. These probes are sensitive to $4.3 \cdot 10^4$ cells/g soil (Holben et al., 1988) and can be constructed to test for the genes encoding hydrocarbon-catabolic pathways (Trevors, 1985).

An important consideration for field work is the enumeration of sessile vs. planktonic bacteria. Attached bacteria are normally more abundant than free-floating cells (Jensen et al., 1985); however, many in situ sampling techniques neglect attached organisms because of the difficulty of retrieving subsurface soil samples from existing monitoring wells.

Microbial enumeration techniques are similar for all scales of observation, but the accuracy of results may not be. Methods which depend on bacterial growth on a laboratory medium such as agar or MPN buffer may not accurately measure in situ activity due to selective growth on the medium (Slater and Lovatt, 1984). The laboratory medium may be free from selective pressures such as contaminant interactions, anoxic conditions and consortia symbiosis which exert a strong influence on adaptation and survival in situ. Caution should be exercised in applying laboratory-scale results to the field scale. Microscale tests can determine the presence of hydrocarbon-degrading organisms at the field scale, but consortium activity is more difficult to quantify.

3.2. Will biotransformation reduce the concentration of contaminant sufficiently?

This question implies the existence of regulatory standards below which contaminant levels must be reduced. Because contaminants often exist in association with each other or with non-contaminant organic carbon, the entire suite of contaminants and alternate substrates must be considered in answering this question. All fractions of petroleum hydrocarbons have been shown to biodegrade in microscale systems under carefully controlled conditions (Atlas, 1981). However, biodegradation may be limited by: (1) failure of the microorganisms to initiate or complete biodegradation due to the lack of a necessary growth factor, (2) incomplete biodegradation due to reduced microbial activity at low substrate concentrations, or (3) the presence of more easily degraded organic carbon which is utilized instead of the contaminants. Theory and laboratory-scale experimental evidence points to the existence of both a threshold concentration below which organisms will not initiate contaminant degradation ($S_{\text{ind}}$), and a limiting concentration below which degradation cannot continue ($S_{\text{min}}$). $S_{\text{ind}}$ can be viewed as the
minimum concentration necessary to induce enzymatic production, and \( S_{\text{min}} \) as the minimum substrate concentration necessary for a net positive energy yield within the cell (Boethling and Alexander, 1979). \( S_{\text{ind}} \) is a function of substrate–receptor interactions within the cell, whereas \( S_{\text{min}} \) takes into account biomass yield and cell decay, as defined by Rittmann and McCarty (1980).

Though \( S_{\text{ind}} \) data exist for chlorinated contaminants, little research has focused on determining possible \( S_{\text{ind}} \) values for non-chlorinated petroleum products. In laboratory-scale experiments, \( S_{\text{ind}} \) for such contaminants as \( p \)-nitrophenol and \( 2,4 \)-dichlorophenoxyacetate (\( 2,4 \)-D) was found to be on the order of micrograms per liter (Boethling and Alexander, 1979; Spain and Van Veld, 1983). Though never measured in the field, \( S_{\text{ind}} \) may be important in situations where the substrate is of limited energy value to the degrading biomass and a low regulatory limit exists.

Experimental data on \( S_{\text{min}} \) are limited because bench-scale experimentation often involves contaminant degradation to below instrument detection limits. \( S_{\text{min}} \) has been measured for benzene at \(< 10 \, \mu g \, L^{-1} \) (Alvarez and Vogel, 1991), 3–5 \( \mu g \, L^{-1} \) (Kuhlmeier and Sunderland, 1985) and \(< 1 \, \mu g \, L^{-1} \) (Jensen et al., 1985) in laboratory experiments. The latter work was performed with alternative sources of contaminant carbon (toluene, xylene, naphthalene, methylnaphthalene and biphenyl) which probably influenced the measured \( S_{\text{min}} \). Degradation of benzene, toluene and \( o-, m-, p- \)-xylenes (BTX) to below 1 \( \mu g \, L^{-1} \) has been demonstrated in the field (Barker et al., 1987), which highlights the possible synergistic influence of both contaminant mixes and non-contaminant organic carbon. These results indicate the site-specific nature of \( S_{\text{min}} \) and the difficulty in measuring it reliably. Both \( S_{\text{min}} \) and \( S_{\text{ind}} \) have been studied exclusively in closed reaction vessels in bench-scale studies. Because the interactions of other contaminant species and alternate carbon sources may influence both enzyme production and cell energy balances, values measured in highly controlled laboratory conditions may not be indicative of field behavior.

Contrary to the above condition, high concentrations of hydrocarbon contaminant substrates may be toxic to microorganisms. Such toxicity has been quantified by the Haldane kinetic model (Folsom et al., 1990), in which bacterial growth declines as substrate concentration increases beyond a finite value (Bailey and Ollis, 1986). Inhibition is typically studied for a single substrate under controlled conditions in a CSTR (continuous stirred tank reactor). Whether laboratory-derived inhibition data translate to the field have not been reported. Like microbial kinetic information, such translation may involve significant interference from alternate substrate sources and a transient consortium. In addition, the presence of a microbial biofilm may afford attached organisms some degree of protection from toxic concentrations of hydrocarbon substrates, as is the case with biocides such as halogen disinfectants (Van der Wende et al., 1989).

3.3. What chemical species limits the extent of biotransformation?

This is primarily a question of reaction stoichiometry and quantification of the initial amounts, rates of utilization and rates of recharge of the reactants within the zone of biotransformation. Stoichiometric equations for aerobic microbial degradation of petroleum hydrocarbons normally include a carbon source (contaminant), oxygen as
electron acceptor, a nitrogen source (NH$_3$ or NO$_3^-$) and a phosphorous source (PO$_4^{3-}$) degrading to biomass, CO$_2$ and water. Excluding trace amounts of phosphorus, the reaction for cell synthesis and energy production from benzene (C$_6$H$_6$) is expressed:

$$C_6H_6 + aO_2 + bNH_3 \rightarrow cCH_{1.8}N_{0.1}O_{0.5} + dCO_2 + eH_2O$$

Cell biomass can be expressed as the molecule CH$_{1.8}$N$_{0.2}$O$_{0.5}$ (Bailey and Ollis, 1986). The amount of O$_2$ necessary to oxidize a given mass of contaminant is determined by the energy yield of the substrate (i.e. how much CO$_2$ is produced relative to biomass) and the conditions of growth. For petroleum hydrocarbons, a single gram of contaminant requires 3–4 g O$_2$ for complete oxidation. Because background levels for dissolved oxygen in groundwater frequently are < 5 mg L$^{-1}$, O$_2$ availability is frequently rate limiting (Barker et al., 1987; Chiang et al., 1989a). Reaction stoichiometry and oxygen yield coefficients are commonly measured through laboratory-scale experiments such as electrolytic respirometry and batch flasks where reactant inputs are carefully controlled, and resultant substrate degradation is accurately measured. Measurement of relative amount of reactants used in the field involves significantly more uncertainty. Dissolved oxygen and nutrient uptake measurements may be subject to large errors due to microbial demand for the degradation of non-contaminant organic carbon and inorganic complexation reactions (Aggarwal et al., 1991). Isolation of microbial consortia in microscale experiments may cause changes in the relative populations of microorganisms, thereby altering the stoichiometric equation and the applicable electron acceptor yield. These error sources notwithstanding, some workers have found laboratory-derived and/or model-predicted stoichiometric and electron acceptor yield data to reflect field-scale behavior, particularly where alternate sources of organic carbon are minimized (Chiang et al., 1989a; Battermann et al., 1994).

The approach of practitioners to site remediation has often been to add essential nutrients in excess with the idea that oversupply will do no harm to the bioremediation process. This strategy has been successful with inorganic nutrients, but is not practical with O$_2$, which is of limited solubility. Attempts to enhance the available oxygen supply through the addition of hydrogen peroxide (H$_2$O$_2$) have been successful; however, high concentrations (100–200 mg L$^{-1}$) of H$_2$O$_2$ may inhibit bacterial metabolism (Brown et al., 1984; Huling and Bledsoe, 1990). Scaling up the addition of H$_2$O$_2$ is subject to greater stoichiometric error than oxygen saturation via air because offgassing of oxygen from peroxide occurs irregularly in groundwater systems. It is therefore difficult to determine how much of the input H$_2$O$_2$ will be available oxygen (Huling and Bledsoe, 1990; Aggarwal et al., 1991).

The issue of limiting chemical species to a reaction is further complicated by the necessity of movement of these species to the reaction site. Laboratory experimentation frequently takes place in stirred flasks where transport of reactants to the reaction site is uninhibited. In field-scale situations, reactant transport and recharge may be dominated by diffusive phenomena, particularly within tight soils. Under diffusion-dominated conditions, reactant flux to the soil–water interface is a function of the bulk solution concentration of the reactant and the diffusivity of the chemical species. Transport properties may therefore limit the availability of essential reactants and hence the extent of biodegradation (Brown et al., 1985; Keely et al., 1986).
3.4. What are the intrinsic kinetics of biotransformation?

Field-scale contaminant removal rates often determine the practical feasibility and ultimate cost of a treatment scheme. Reaction kinetics determine how quickly a microbial community will degrade a contaminant to acceptable levels. Larson (1984) lists three areas of impact of kinetic data: (1) kinetics-based biodegradation rate expressions are integral to computer models of degradation; (2) kinetic data are necessary to extrapolate degradation results from one environmental compartment to another (also from one scale to another); and (3) kinetic data provide an objective basis for evaluating the relative persistence of a chemical in a particular environment. From the perspective of effective scale-up, of primary importance are the reliability of laboratory-scale kinetic constants as predictors of field-scale behavior, and whether the degradation kinetics of one contaminated site offer a reasonable approximation of those at a similar site.

Much microscale research has focused on the determination of single species or consortia kinetics for growth on a single contaminant compound in aqueous or soil slurry microcosms. The results of these studies show wide variation in removal rates for similar compounds using different microbial inocula (Fig. 2). Most researchers report kinetic data either in the form of mass of contaminant removed per biotic mass per day [e.g., mg toluene (mg cells)$^{-1}$ (day)$^{-1}$] or, in the case of aqueous systems mass of contaminant removed per volume per day (mg toluene L$^{-1}$ day$^{-1}$). Where biotic mass

![Fig. 2. Observed degradation rate dependence of aromatic hydrocarbons (BTEX) on initial substrate concentration under aerobic, denitrifying, and methanogenic conditions. All points were measured in the laboratory.](image)
is not reported, comparison with other research becomes impossible, and the rates reported are valid only for a specific site. In studying alkylbenzene degradation in aqueous microcosms under low oxygen conditions, Armstrong et al. (1991) found toluene from a landfill leachate plume degraded at a rate of 1.2 \( \mu \text{g L}^{-1} \text{ day}^{-1} \), and Barker et al. (1987) measured BTX degradation at 27–52 \( \mu \text{g L}^{-1} \text{ day}^{-1} \). Soil slurry microcosms using similar contaminants and concentrations under higher dissolved oxygen conditions have found much higher degradation rates, up to 50 \( \text{mg L}^{-1} \text{ day}^{-1} \) (Alvarez and Vogel, 1991; Hutchins, 1991). The variation between these observations is undoubtedly due in part to inocula concentration differences as well as the dissolved oxygen regime, but such data underscore the need to match experimental conditions with aquifer conditions. Other work has indicated that microcosms which only consider the biota suspended in the groundwater may underestimate the biodegradation potential of an aquifer. In evaluating the importance of attached (biofilm) vs. suspended (planktonic) microbial growth, Jensen et al. (1985) reported that attached cells degraded naphthalene at lower rates than planktonic cells, though of the same order of magnitude. While most soil systems are thought to contain many more attached than suspended cells, the latter may be disproportionately responsible for contaminant removal.

Reaction order is also subject to some variability, though most researchers show biodegradation following either zero-, first-, or some fractional-order dependence, which is to be expected from the saturation kinetic model (Jensen et al., 1985; Barker et al., 1987; Major et al., 1988; Hutchins, 1991; Hutchins et al., 1991b). Jensen et al. (1985)

Fig. 3. Observed degradation rate dependence of aromatic hydrocarbons (BTEX) on initial substrate concentration under laboratory and field measurement conditions. All points were measured under aerobic or denitrifying conditions.
reported a very low half-saturation coefficient for naphthalene degradation \( K_s = 0.1-1.0 \ \mu g \ L^{-1} \). If this is representative of in situ consortia degrading aromatics, then zero-order kinetics should be expected for these compounds at the concentrations commonly found at contaminated sites. Barker et al. (1987) point out that the geometry of a contaminant plume may be an important factor in determining both the rate and order of contaminant removal. Zero-order kinetics were observed for BTX degradation in soil microcosms but mass loss observed in the field appeared to follow first-order kinetics. This suggests that reaction order in situ may be controlled by some other process. The authors speculate this to be oxygen availability in the aquifer. Where plumes have little surface area exposed to oxygenated groundwater, a relatively small fraction of the plume may contain active microorganisms. Chiang et al. (1989a) also observed field-scale degradation kinetics to be oxygen limited. This limitation is the rule rather than the exception in most aquifers, but the process of oxygen diffusion to the reaction site is not represented in most microcosm studies. In scaling up kinetic values, the transport limitation of electron acceptor to the reaction site must be considered. Alternate electron acceptors which exhibit higher water solubilities, such as nitrate, will be more available than oxygen under transport-limiting conditions if they are supplied in adequate quantities.

The extrapolation of kinetic rates between observational scales is necessary to employ an engineering approach to site remediation. The potential for major discrepancies in these rates is high, particularly where contamination exists in a concentrated plume or a nonaqueous-phase liquid (NAPL). Successes in micro- to macroscale rate prediction have been achieved (Chiang et al., 1989a) through consideration of as many of the macroscale factors as possible. Fig. 3 shows a compilation of BTEX (benzene, toluene, ethylbenzene and \( o-,m-,p- \)-xylenes) degradation rates plotted as a function of the initial substrate concentration. Separate plots are shown for laboratory- and field-scale remediation systems. The roughly linear progression of laboratory-scale rates is predicted by Monod kinetics, where \( S \) in on the order of \( K_s \); however, there is an apparent stagnation of field-scale degradation rates as concentration increases.

4. Mesoscale phenomena

4.1. Does sorption influence the rate of bioremediation?

Research focusing on hydrocarbon sorption to soils is detailed and extensive, resulting in numerous isotherm models which predict adsorbed-phase concentration as a function of aqueous-phase concentration. The issue of bioavailability of sorbed solute has received much less attention. Much research focuses on whether sorbed solute can be biodegraded by either sorbed or planktonic bacteria, and whether solute desorption kinetics (mass transfer of contaminant from the sorbed phase to the liquid phase) control the rate of biodegradation. Researchers have postulated both positive and negative answers to both questions, suggesting that experimental conditions may be of prime importance. Such factors as solute hydrophobicity and sorptive properties, soil micro-
porosity and organic matter fraction, biotic concentrations and characteristics, and agitation of the experimental apparatus may all play roles in the bioavailability of sorbed solute and desorption kinetics.

Most research addressing the issue of sorbed solute bioavailability has concluded that sorbed hydrocarbon is not available for biodegradation (Steen et al., 1979; Knezovich and Harrison, 1987; Smith et al., 1992). In modeling the coupled processes of sorption and biotransformation in column experiments, Angley et al. (1992) concluded that biotransformation occurs only in solution and that desorption was generally diffusion-rate limited. Ogram et al. (1985) proposed three models to describe contaminant biodegradation in soil. The most successful model assumed that both attached and planktonic bacteria were capable of utilizing dissolved contaminant, but neither was able to degrade sorbed contaminant either because contaminant may have been adsorbed sufficiently deeply within the soil–organic matter matrix that bacteria were unable to reach it, or because enzymes responsible for degradation may be incapable of attacking sorbed contaminants.

Several researchers have observed biodegradation of sorbed contaminants via attached organisms in sediments and granular activated carbon (Remberger et al., 1986; Speitel and DiGiano, 1987). This suggests that the attached organisms may enhance contaminant concentration gradients, promoting desorption directly into the biofilm. A highly adsorptive surface such as activated carbon may also offer a degree of protection from the toxic effects of high aqueous-phase contaminant concentrations. Ehrhardt and Rehm (1985) found attached cultures of *Pseudomonas* sp. to be significantly more resistant to high phenol concentrations than planktonic cells. Rapid contaminant adsorption by activated carbon, and subsequent release to the biofilm had a stimulating effect on the attached biota.

In an attempt to address the question of whether desorption limits biodegradation, Mihelcic and Luthy (1991) coupled the model of Wu and Gschwend (1986) with Michaelis–Menten biodegradation kinetics to predict the desorption and biodegradation of naphthalene in a soil system with aggregate micropores. Results indicated that the naphthalene adsorption/desorption process was rapid compared to biodegradation and therefore did not limit substrate utilization. Using a similar model, Rijnaarts et al. (1990), modeled biodegradation in a mixed soil system which was desorption limited. In this case contaminant diffusion through the soil micropores was found to limit biodegradation rate. In microscale experiments, Bouwer et al. (1994) found naphthalene biodegradation proceeded more slowly in high organic carbon systems, suggesting desorption rate limitations.

Contradictory results are not unexpected considering the potential for differences in the systems studied. Many contaminant, soil and microbial consortium variables effect the desorption–biodegradation co-process. Solute hydrophobicity, solute diffusivity, equilibrium partitioning coefficient, soil organic matter content, soil particle geometry, soil porosity, soil moisture content, microbial kinetics, and the presence or absence of a biofilm may all influence either the desorption or biodegradation rates. The effects of soil moisture content on sorption and biotransformation were illustrated by Estrella et al. (1993). In comparing the results of 2,4-D desorption–biodegradation experiments in saturated and unsaturated soil columns, the authors found that biodegradation rate
and soil–water partitioning coefficient were significantly higher in unsaturated systems.

Another possible reason for contradictory conclusions in desorption limitation experiments may be the description of adsorption/desorption as a two-stage process. In studying toluene degradation in an organic soil, Robinson et al. (1990) reported the first stage reflects the rapid adsorption/desorption of ~ 90% of the toluene, while the remaining ~ 10% adsorbs/desorbs more slowly. The significance of the two apparent rates is that the microbial degradation reaction was limited by the slow desorption rate. In validating a model to predict organic compound transport and sorption in non-ideal (heterogeneous) systems, Brusseau et al. (1992b) was able to predict contaminant breakthrough for four different aquifer systems by representing sorption as a dual process. Sorption was modeled to be instantaneous for a portion of the aquifer material while being diffusion rate-limited for the remainder. This may be explained by mineral surface sorption (fast) vs. organic matter sorption (slower). Adsorption to inorganic particles (surface adsorption) becomes appreciable at soil organic matter contents of < 0.1% (Pignatello, 1989).

Interactions of soil organic matter amount and composition, contaminant hydrophobicity, and the length of time the contaminant has been in place make generalizations about sorptive behavior difficult. Organic polymers within the soil attract organic contaminants via their nonpolar gel structure. Once within the polymer matrix, contaminants may bond (covalent or hydrogen bonding) to specific sites, or may diffuse through micropores in the gel. The longer the exposure time to the contaminant, the greater the probability that contaminant bonding and deep-matrix diffusion will occur (Pignatello, 1989). Pignatello further suggests that if such tightly bound contaminants desorb at all, it is at a rate which certainly limits biodegradation. In studies with naphthalene, Guerin and Boyd (1992) found desorption rate limitations to be organism specific, which suggests that the bioavailability of contaminants may also vary depending on the consortia present.

To make sense of the array of variables influencing sorption and employ an engineering approach to answering the question of whether desorption limits biodegradation in any particular situation, it may be advantageous to formulate a Thiele-type modulus which compares the variables of desorption with those of biodegradation in a dimensionless number. Traditionally, a Thiele modulus compares the diffusive flux of substrate to the rate of reaction. The modification necessary here would be to substitute the desorptive flux of substrate for diffusive flux. This type of comparison could be very helpful in determining the effects of scale-up on desorption–biodegradation limitations.

Progress in the area of sorptive behavior has been considerable, however, the above citations indicate the need for further description of the soil-, organism- and contaminant-specific interactions of sorbed solute bioavailability and desorption kinetics. In a recent review, Fogarty and Tuovinen (1991) concluded’‘In general, sorption isotherms in relation to contaminant bioavailability to microorganisms are poorly understood and have yet to be experimentally addressed’’.

Sorptive phenomena in the field are usually expressed in terms of a retardation factor, which can be described as the ratio of the rate of movement of a non-sorbing species to the rate of movement of the contaminant. Retardation factors are modeled as a function (μmax) of soil properties.
of partition coefficient \((K_d)\), soil bulk density and porosity. The conditions necessary for accuracy in such a model are (Lee et al., 1988): (1) adsorption isotherms must be linear relationships between solute and solid-phase concentration; (2) reaction kinetics must be fast relative to the advective flux of contaminant via groundwater flow; (3) reactants and binding sites within the aquifer must be uniformly distributed; and (4) contaminant transport must be independent of other liquid phases. The retardation model requires significant simplifications and aquifer assumptions which are usually present only in highly homogeneous aquifers. For this reason, most macroscale sorptive work has been done in uniform sandy soils, free from clay lenses or excessive organic matter. Some recent models, such as that presented by Brusseau (1992) alleviate the need for condition (2) above by treating sorption as a two-stage process. Such models advance the understanding of sorption processes through phenomenological, rather than simply empirical, accuracy.

The question of whether laboratory sorption experiments yield information useful to field remediation research has not been adequately addressed. Sorptive behavior is the result of the complex interactions of contaminant, soil and biotic characteristics. More highly controlled field-scale experimental work and further incorporation of field heterogeneities into available models is necessary to determine which characteristics are most important in the field, and which laboratory techniques best represent them.

4.2. Does interphase transport limit the rate of bioremediation?

Petroleum products spilled in a soil system can exist in four phases: (1) a non-aqueous-phase liquid (NAPL) either free floating on the water table or bound to soil particles; (2) dissolved in soil water (aqueous phase); (3) vapor phase existing in soil pores in the unsaturated zone; and (4) adsorbed to soil particles or soil organic matter. Most petroleum products exhibit low solubilities in water, facilitating the formation of free-floating NAPL which may move slowly downgradient, but acts essentially as a stationary source of contaminant recharge to the groundwater. If soil conditions permit, the NAPL will also act as a source of contamination for the vadose zone, as the more volatile hydrocarbon fractions move into the vapor phase. Biodegradation of subsurface hydrocarbons occurs in the aqueous phase, either below the capillary fringe or in hydrated microsites within the vadose zone (Morgan and Watkinson, 1989). Hydrocarbon movement from the NAPL or sorbed phase to the aqueous phase is therefore a prerequisite for biodegradation to occur. If these processes occur at a slower rate than site microorganisms are capable of utilizing the contaminant, interphase transport will limit biodegradation.

Also of concern is the movement of contaminant from micropores within the soil matrix, which may exclude microorganisms, to macropores where organisms are present. Though technically within the same phase, transport between these domains has the effect of moving contaminant from an unavailable to an available state, and therefore may limit biotransformation. Scow and Alexander (1992) reported such pore-domain limitations in studying the effects of clay aggregates on biodegradation kinetics of phenol and glutamate. The existence of multiple pore domains where transport is inhibited may furthermore lead to non-equilibrium conditions with respect to both sorbing and non-sorbing solutes, as modeled by Brusseau et al. (1992a).
For sparingly soluble compounds such as long-chain alkanes and poly-aromatic hydrocarbons, contaminant degradation rates in aqueous solution are often limited by the rate of dissolution from the NAPL to the aqueous phase (Thomas et al., 1986; R.M. Miller and Bartha, 1989). Lower-molecular-weight hydrocarbons and alkylbenzenes exhibit much higher solubilities, and therefore dissolve into the aqueous phase more rapidly.

Though macroscale research is not plentiful, mass transport limitations evident at the microscale may be magnified in many field-scale situations, particularly in soils with fine texture and low porosity. In such situations, advective transport may be minimal, further slowing the dissolution process. Macroscale features such as soil type and porosity will also affect the NAPL surface area/volume ratio. As dissolution is a linear function of interphase surface area, such features may determine whether dissolution limits biodegradation (Spain and Van Veld, 1983; J.T. Wilson et al., 1989). J.T. Wilson et al. (1989) further emphasize the need for accurate quantification of the nonaqueous phase both for electron acceptor demand calculations and for estimating the contaminant recharge to the groundwater and vapor phases. Where macroscale research has been performed, results are predictably site-dependent. Thomas et al. (1986) observed octadecane mineralization to be dissolution controlled, while naphthalene dissolution was found to be faster than biodegradation (Foght and Westlake, 1982).

Efforts to increase the rate of contaminant dissolution from a NAPL to the aqueous phase have centered on the addition of surfactants to soil systems. Such surfactants have been used extensively in marine oil spill treatment for many years and have recently been used in terrestrial applications as well (Hoeppel et al., 1991). Site microbial communities may also be responsible for the production of surfactants to speed contaminant dissolution (Vanloocke et al., 1979; Bossert and Bartha, 1984). Whether NAPL dissolution limits biodegradation can be measured in the laboratory, but the result obtained may not be representative of the field scale unless the site biotic, soil and NAPL characteristics were fully considered in the microscale experimentation.

5. Macroscale phenomena

5.1. Does advective-dispersive transport limit the rate of biodegradation?

Mass transport by advection and dispersion have a significant effect on contaminant distribution and substrate/electron acceptor availability to microorganisms. Field experimentation indicates oxygen transport limitations cause contaminant degradation rates at the edge of a subsurface plume to be much higher than those in the plume center due to the maintenance of aerobic conditions at the edge (Chiang et al., 1989a; Morgan and Watkinson, 1989). Such transport limitations apply to nutrients and other electron acceptors as well, but are most often observed with oxygen due to its common stoichiometric limitation. This is usually not observed at the microscale, where mass transport limitations are often minimized or eliminated by design.

Several workers have found evidence for advective transport limitations to biodegradation at the microscale. Limitations are most apparent at zones of contact between strata which differ significantly in their ability to conduct fluid flow. Field evidence
indicates contaminant persistence in areas of lower advective flow velocity or hydraulic conductivity (Sutton and Barker, 1985; Barker et al., 1987; Chiang et al., 1989a). The effects of advective and dispersive transport on biodegradation rates are becoming more evident in the modeling literature as well. To illustrate the rapid progress in this field, Lee et al. (1988) noted that although then current models did take into account the changes in solute (contaminant and electron acceptor) concentrations resulting from differences in aquifer permeability, no modeling efforts had sought to relate these variations to the inevitable effect they must have on the rate at which bioremediation takes place in situ. More recent modeling efforts (MacQuarrie and Sudicky, 1990) have illustrated that variation in aquifer physical characteristics such as dispersivity can significantly affect advective flow and rates of biotransformation over comparatively small spatial separations.

The overall effect of increasing advective-dispersive transport within an aquifer is to enhance the process of mixing contaminants, electron acceptor and cells capable of contaminant biodegradation. Only where all three of these constituents are present concurrently can biodegradation occur. Lee et al. (1988) observe that this mixing is frequently confounded in aquifers in which contaminants adhere to solids within areas of low advective flow, while dissolved oxygen and nutrients flow within adjacent higher-permeability zones.

5.2. How does spatial heterogeneity impact bioremediation?

Spatial heterogeneity at contaminated field sites can significantly influence contaminant movement and rate of degradation. Subsurface properties which are subject to significant spatial variation include porosity, permeability, degree of microbial colonization, and chemical properties such as nutrient and electron acceptor conditions. If significant heterogeneities exist, different phenomena may limit the rate and extent of biodegradation across the site. For example, an often encountered soil textural heterogeneity is the presence of clay lenses in otherwise permeable sandy soils, which can reduce the local permeability by up to 5 orders of magnitude (Todd, 1980). As mentioned above, drastically reduced groundwater flow velocities generally make diffusive transport predominant within clay lenses. This may cause such lenses to act as reservoirs for contaminants, recontaminating groundwater for long periods after more permeable zones have been cleansed. Under these conditions biotransformation within the clay could be limited by molecular diffusion while the higher-permeability zones may be limited by advection.

Physical heterogeneities can also cause significant chemical heterogeneities, such as the establishment of various redox zones within the aquifer. While aerobic biotransformation may occur within advective-flow dominated areas, oxygen may be absent where diffusion is the primary transport mechanism. Under these circumstances, nitrate, sulfate, iron (III) and carbon dioxide may be sequentially utilized as electron acceptors. As the most energetically favorable alternative to oxygen, hydrocarbon degradation using nitrate as the electron acceptor has been extensively studied. Laboratory research has shown most petroleum hydrocarbons biodegrade under denitrifying conditions. Though several researchers report success (Werner, 1985; Major et al., 1988), many
studies indicate benzene, typically the petroleum hydrocarbon of greatest regulatory concern, remains recalcitrant under denitrifying conditions (e.g., Kuhn et al., 1988; Hutchins, 1991; Hutchins et al., 1991b). Denitrification has been used to successfully biodegrade jet fuel at the field scale (Hutchins et al., 1991a), though the authors found that ~10 times as much nitrate was utilized as could be accounted for by contaminant degradation alone. Another scale effect noted was the relative recalcitrance of sorbed JP-4 fuel oil within the aquifer.

Aromatic compound degradation has also been observed under iron-reducing (Lovley and Lonergan, 1990) and methanogenic (B.H. Wilson et al., 1986) conditions in laboratory experiments. However, there has been little field-demonstration work with these less energetically favorable electron acceptors. Though biodegradation reactions using alternative electron acceptors occur at a much slower rate than under aerobic conditions, these reactions may contribute significantly to overall contaminant removal in the field. The practitioner should therefore be aware of the redox conditions of the contaminated zone such that the presence of alternative electron acceptors can be exploited.

Contaminants also may exhibit significant spatial heterogeneity with regard to flow channels and contaminant phase. An example is the weathering process of a spilled gasoline or crude oil, where the lighter hydrocarbon fraction may volatilize quickly after introduction, leaving progressively heavier fractions down the path of migration. In addition, dispersive activity and the progression of biodegradation from the edges into the center of a plume usually cause the plume to contain significantly higher (≥3 orders of magnitude) contaminant concentrations in its center than at the edges, causing differences in the rate as degradation proceeds. Where biodegrading microorganisms are primarily fixed to aquifer solids, the residence time of the contaminated groundwater in the system must be sufficient to allow the reaction to proceed to completion. Aquifer physical heterogeneities which effect groundwater flow rates therefore may impact degradation rates.

The effects of nutrient conditions in soil and aquifer system petroleum degradation has been studied and reviewed extensively; however, consideration of the impact of spatial heterogeneities on nutrient availability has not. This issue is important mainly in nutrient-poor aquifers where the addition of nutrients is conducted via injection or surface application. Added nutrients must flow to the site of active microorganisms and therefore are subject to transport limitations imposed by aquifer heterogeneities.

Also germane to the discussion of spatial heterogeneities is the phenomenon of localized plugging of a subsurface formation, creating zones of very low permeability and significantly reduced transport of oxygen and nutrients. Such plugging has been observed from a variety of unrelated sources, such as the formation of gas bubbles, the accumulation of microbial biomass, or chemical precipitation within soil pores. Microbial plugging of aquifers has been demonstrated in laboratory systems with porosity and permeability decreases of up to 96% and 98%, respectively (Taylor and Jaffe, 1990; Cunningham et al., 1991). Bacteria injected into model core systems have been observed to attach close to the point of injection and plug the core due to copious exo-polysaccharide production (Shaw et al., 1985; Raiders et al., 1986; Torbati et al., 1986). Using starved bacteria, MacLeod et al. (1988) found injected cells to be uniformly distributed
in artificial rock cores, and subsequently found significant permeability reductions when growth conditions were enhanced (Lappin-Scott et al., 1988).

Hydrogen peroxide is frequently added to augment the dissolved oxygen supply in \( \text{O}_2 \)-poor groundwater. \( \text{H}_2\text{O}_2 \) is more water soluble than molecular oxygen, allowing much greater oxygen transport to the subsurface; however, \( \text{H}_2\text{O}_2 \) rapidly reacts to form elemental oxygen and water. As discussed earlier, liberated oxygen may form bubbles in the aquifer, decreasing permeability and preventing transport (Thomas et al., 1987; J.T. Wilson and Ward, 1987; Pardieck et al., 1992). Aquifer plugging due to precipitation of inorganic insoluble salts is well documented, particularly where biodegradation in the aquifer leads to oxygen-poor conditions.

The addition of oxygen- and phosphate-enriched nutrient solutions may alter the soil–groundwater chemical equilibrium to the point of precipitation of Fe-, Mn- and Ca-phosphates (Aggarwal et al., 1991). Biomass buildup and inorganic precipitation may occur concurrently, exacerbating plugging problems. Microbial production of extracellular polysaccharides may entrap precipitates, further decreasing aquifer permeability.

6. Overall issues

6.1. How can bioremediation rates be evaluated and predicted?

Hydrocarbon removal from a contaminated site may occur for a variety of reasons. Volatilization, off-site migration, sorption onto soil particles and abiotic transformation make biodegradation a difficult process to prove in situ. The highly controlled conditions of the microcosm allow substantiation of biodegradation claims, but the addition of unmeasurable contaminant sinks in field-scale systems have led researchers to the use of a variety of site characteristics to corroborate contaminant disappearance data. Madsen (1991) suggests an approach to proving biodegradation which integrates chemical factors such as selective disappearance of microbiologically labile isotopes or nonreactive tracers with biotic factors such as increased activity in degrader microorganisms or predators which feed upon them.

Many researchers have relied on contaminant disappearance and concurrent hydrocarbon-degrader population increases to prove biodegradation. The most common laboratory technique is the use of \(^{14}\text{C}\)-labelled contaminants with a known microbial consortia. Microcosms using native microbial populations have shown good correlation with the disappearance of crude oil (Mulkins-Phillips and Stewart, 1974), jet fuel (Song and Bartha, 1990; Hutchins et al., 1991a), aromatics (Arvin et al., 1989; Karlson and Frankenberger, 1989) and phenolics (Spain et al., 1980) under aerobic and anaerobic conditions. Several researchers have suggested the measurement of protozoan predators as a useful indicator of bacterial vigor (Rogerson and Berger, 1981; Madsen et al., 1991).

The transition from the laboratory to the field necessarily involves the use of several concurrent confirmatory tools to prove bioremediation is responsible for contaminant removal. Several field-scale studies have shown both increased bacterial numbers and contaminant removal in crude oil; however, more macroscale work has focused on
linking contaminant removal with electron acceptor disappearance (Barker et al., 1987; Chiang et al., 1989a). Recent research has sought to determine in situ biodegradation rates through correlation with O$_2$ removal and CO$_2$ production in bioventing applications (Hinchee et al., 1991) or through observed changes in groundwater chemistry brought about by the biotransformation reaction (Rifai and Bedient, 1994; Wiedemeier et al., 1994). The choice of methods to monitor and verify bioremediation is dependent on the level of control which may be imposed upon the experimental system. Field sites will always involve uncertainty, the challenge therefore is to prove bioremediation through a battery of methods which, taken together, offer compelling evidence that microorganisms are responsible for the observed contaminant disappearance. Several methods demonstrating bioremediation are listed in Table 5.

6.2. How does temperature influence the rate of bioremediation?

Although temperature is usually beyond the control of the practitioner, it remains valuable to understand how diurnal and seasonal changes in temperature impact bioremediation performance. In addition, in certain cases, such as covered land farm operations, the practitioner may be able to exert some influence on the temperature. From a theoretical perspective, temperature can be expected to influence process rates at every scale of observation; however, such factors as microbial origin and season of collection have been shown to be important considerations when evaluating the effects of temperature in laboratory studies. It is well documented that mixed cultures fed petroleum hydrocarbons are most efficient at between 20° and 30°C (Dibble and Bartha, 1979; Atlas, 1981; Song et al., 1990). Bacterial consortia are being freshly removed from the environment; however, they utilize organics fastest when incubated at the ambient temperature from which they were removed (Morgan and Watkinson, 1989; Atlas, 1981). Furthermore, biotic samples from the same site taken at different times of year show differences in species predominance. Psychrophilic organisms may predominate in winter months, while not measurable in the summer. Several researchers have reported the maintenance of contaminant removal at very low temperatures in situ (Morgan and Watkinson, 1989), though microbial activity does appear to cease in frozen soils.

The effects of temperature on bioventing in the vadose zone may be more pronounced than in saturated-zone biodegradation because greater temperature fluctuations typically occur closer to the surface. Several bioventing studies performed at U.S. Air Force bases have indicated Arrhenius-type dependence of biodegradation rates on soil temperatures (R.N. Miller et al., 1991; Ong et al., 1994). In studying cold-climate bioventing at several sites in Alaska, Ong et al. (1994) observed several instances of biodegradation rates on the order of 20 mg kg$^{-1}$ day$^{-1}$ for mixtures of JP-4 and heavier oils at temperatures $< 15^\circ$C. Using similar measurement techniques at temperate sites, Hinchee and Ong (1992) found much lower biodegradation rates (2–8 mg kg$^{-1}$ day$^{-1}$) for similar contaminants at higher temperatures (17–25°C). Prediction of bioventing performance based on temperature alone is clearly not appropriate.

The net effect of temperature on the overall rate of bioremediation depends on which processes are limiting. To illustrate the possible effects of temperature on bioremedia-
tion, Fig. 4 compares the predicted relative temperature dependency of microbial growth, molecular diffusion and advective solute transport. Increasing the temperature generally increases process rates of growth, diffusion and advection. It is therefore important to consider not only the response of the site consortium to temperature changes but also the effects temperature changes may have on other phenomena important to the bioremediation reaction (such as diffusive and advective transport).

<table>
<thead>
<tr>
<th>Demonstration model</th>
<th>Reliability/problems</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminant attenuation</td>
<td>laboratory: reliable, depending on controls; volatilization often a problem</td>
<td>among many others: Barles et al. (1979), Dibble and Bartha (1979),</td>
</tr>
<tr>
<td></td>
<td>field: not usually reliable, often many uncontrolled abiotic loss mechanisms</td>
<td>Kilbane et al. (1983)</td>
</tr>
<tr>
<td>Electron acceptor loss</td>
<td>laboratory and field: may indicate non-contaminant organic matter is degrading</td>
<td>Zeyer et al. (1986), Chiang et al. (1989b), Hutchins et al. (1991a)</td>
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<td></td>
<td>(particularly in the presence of enhanced N, P); adequate controls often not possible in the field</td>
<td></td>
</tr>
<tr>
<td>$^{14}$CO$_2$ capture</td>
<td>laboratory: usually the most reliable indicator of mineralization if good controls are used</td>
<td></td>
</tr>
<tr>
<td></td>
<td>field: not practical for use in field situation—capture of CO$_2$ very difficult</td>
<td>among any others: Pfaender and Bartholomew (1982), Spain and Van Veld (1983), Kuhn et al. (1988)</td>
</tr>
<tr>
<td>Growth of contaminant degrading microbes</td>
<td>laboratory and field: offers reliable supporting evidence of biodegradation, though not conclusive proof alone, especially at field scale</td>
<td>Wyndham and Costerton (1981), Spain and Van Veld (1983)</td>
</tr>
<tr>
<td>Nutrient removal (N, P)</td>
<td>laboratory: reliable indicator of biotic activity where not completing substrates and adequate controls are used</td>
<td></td>
</tr>
<tr>
<td></td>
<td>field: supporting evidence only; not reliable where alternate substrates are available</td>
<td>Jamison et al. (1975), Odu (1978), Swindoll et al. (1988)</td>
</tr>
<tr>
<td>Intermediate metabolite production</td>
<td>laboratory and field: reliable indicator of biodegradation; controls to insure metabolite was not indigenous to the site are necessary</td>
<td>Dasappa and Loehr (1991)</td>
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<tr>
<td>Degradable/non-degradable contaminant ratios</td>
<td>laboratory and field: most common is the C$_{17}$/pristane ratio; reliability hinges on pristane persistence in medium</td>
<td>J.T. Wilson et al. (1989), Pritchard and Costa (1991)</td>
</tr>
<tr>
<td>Growth of non-contaminant degrading microbes</td>
<td>laboratory and field: growth of protozoan grazers has been used as an indirect measure of bacterial growth; reliability compromised by protozoan feeding on non-contaminant degrading bacteria</td>
<td>Madsen (1991)</td>
</tr>
</tbody>
</table>
6.3. How can bioremediation be modeled?

Numerous mathematical models have been proposed with application to in situ bioremediation. Such models integrate processes occurring at different scales of observation to predict spatial and temporal patterns of contaminant distribution. Table 6 summarizes phenomenological models of bioremediation, focusing on studies published in the last decade. Common to these models is advective-dispersive transport of a dissolved contaminant. Equilibrium partitioning of the contaminant between the aqueous and solid phases is usually assumed. Reaction is handled by a wide variety of rate expressions, the most common being first-order, Monod and double Monod kinetics. About half of the models combine material balances on the electron acceptor and biomass in addition to the substrate. Those models that consider only the substrate lack the ability to describe limitation by an electron acceptor, or to account for the change in intrinsic reaction rate that surely accompanies biomass growth or decay. Since electron acceptor limitation is believed by many to be common, failure to account for electron acceptor transport and reaction would appear to restrict model applicability. An aspect of bioremediation modeling that is poised for further development is description of interphase transport and/or non-equilibrium sorption. These processes are contained in only ~ 20% of the models shown in Table 6. A review of model development for these processes and the extent to which they have been coupled for porous media systems can

![Graph showing temperature dependency of constituent processes of bioremediation.](image_url)
## Table 6
### Phenomenological models for in situ bioremediation

<table>
<thead>
<tr>
<th>Reference(s)</th>
<th>Transport a</th>
<th>Kinetics b</th>
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<th>Sorption d</th>
<th>Phases e</th>
<th>Data</th>
<th>Other</th>
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<td>van Genuchten (1981)</td>
<td>1-D</td>
<td>S</td>
<td>E</td>
<td>D</td>
<td>–</td>
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<tr>
<td>Enfield et al. (1982)</td>
<td>1-D</td>
<td>S</td>
<td>E</td>
<td>D</td>
<td>–</td>
<td>field</td>
<td></td>
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<tr>
<td>Sykes et al. (1982)</td>
<td>2-D</td>
<td>M</td>
<td>SM</td>
<td>–</td>
<td>D</td>
<td>field</td>
<td></td>
</tr>
<tr>
<td>Borden et al. (1984)</td>
<td>2-D</td>
<td>DM</td>
<td>SEM</td>
<td>E</td>
<td>D</td>
<td>–</td>
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<td>Semprini and McCarty (1991a,b)</td>
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<td>Brusseau et al. (1992a)</td>
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</table>
be found in Brusseau et al. (1992b). Some of the other phenomena that have been occasionally incorporated in models include diffusive transport between the bulk liquid and a surface-associated biological phase (Molz et al., 1986; Widdowson et al., 1988; Baveye and Valocchi, 1989), oxygen transfer from the unsaturated to the saturated zone (Borden and Bedient, 1986; Borden et al., 1986; Rifai et al., 1988), hydrologic spatial heterogeneity (Dawson et al., 1986a,b; Wheeler and Dawson, 1987; Chiang et al., 1989b; MacQuarrie and Sudicky, 1990; Dawson and Wheeler, 1992;) and microbial transport (Corapcioglu and Haridas, 1984; Borden and Bedient, 1986; Wheeler et al., 1987).

Of the models cited in Table 6, only a handful involve comparison of simulations with field-measured gradients in space (Enfield et al., 1982; Sykes et al., 1982; Huyakorn et al., 1985; Borden et al., 1986; Rifai et al., 1988) or time (Wagenet and Hutson, 1986; MacQuarrie and Sudicky, 1990; Semprini and McCarty, 1991a; Semprini and McCarty, 1991b). Only the BIOPLUME model has been applied to more than one site (Borden et al., 1986; Rifai et al., 1988; Chiang et al., 1989a). Further field testing should be a priority, even though this involves considerable expense and labor. Broad evaluation of models by comparison with laboratory and field data will be required to confirm that their structure incorporates all essential processes.

7. Feasibility issues

Decision makers must be aware of the phenomena which influence bioremediation and the ways in which these phenomena interact. At the microscale, reaction kinetics are the primary concern. At the mesoscale, the partitioning processes of sorption and interphase transport are of primary interest. At the macroscale, flow-related processes of advection and dispersion, along with the effects of field heterogeneities can influence the rate and extent of in situ bioremediation. Assessment of feasibility of an in situ bioremediation project is dominated by the need to identify and estimate the appropriate

Notes to Table 6:

- Spatial dimensionality: 1-D, 2-D, 3-D = one-, two-, and three-dimensional, respectively.
- 1 = first order; M = Monod; DM = double Monod; TM = triple Monod; F = instantaneously fast reaction between substrate and electron acceptor; I = inhibition; C = cometabolism.
- S = substrate (contaminant); E = electron acceptor or nutrient; M = microorganisms.
- E = equilibrium sorption; NE = nonequilibrium sorption; = not considered.
- D = dissolved; I = insoluble; G = gaseous.
- Oxygen transfer from the unsaturated to saturated zone.
- Theoretical development.
- Microbial transport.
- External mass transfer between bulk liquid and biological phases.
- Multiple substrates or metabolites.
- Two-zone model.
- Multiple microorganisms.
- Internal mass transfer resistance within a biofilm.
- Water loss by plant transpiration.
- Spatial heterogeneity in hydraulic conductivity.
rate-controlling phenomena. A mechanism for this assessment is to determine if and at what rate biotransformation is occurring at the site without intervention. Observation of intrinsic bioremediation is subject to field-scale measurement difficulties, but can offer valuable information on potentially limiting factors. In cases where contaminant exposure risk is low, passive remediation may occur at rates adequate to convince regulatory agencies that the expense of an engineered system is unnecessary (Wiedemeier et al., 1994). Passive remediation alone is most likely to be successful at sites where mass transport limitations are minimized, such as in highly conductive aquifers and with non-recalcitrant substrates (Barker et al., 1987; Borden and Bedient, 1987). Determination of naturally occurring biodegradation is typically made during initial assessment of a contaminated site. Data gathered should both document contaminant reduction and provide compelling evidence (via mass-balance calculations) that biodegradation is the responsible mechanism (Wiedemeier et al., 1994).

8. Technology development needs

The volume of current bioremediation literature is dominated by investigation of individual phenomena (usually at the micro- or mesoscale). Relatively few studies address interactions among different bioremediation phenomena or consider how phenomenological effects can be integrated to make predictions of field-scale process behavior. The relative absence of practitioner-oriented tools for decision making suggests that a process engineering approach is needed to improve the state of the art of bioremediation practice. Process engineering, in the context of bioremediation, involves the integration of site historical information, site geologic, hydrologic, chemical and microbiological characteristics, laboratory and field data, and possible remedial actions in order to make predictions and design decisions. Performing this integration is the ultimate and arguably the most difficult step of the scale-up process. The approach currently followed by most practitioners of bioremediation relies heavily on the results of bench-scale treatability studies and pilot- or field-scale demonstrations (B.H. Wilson et al., 1986; Rogers et al., 1993). This experimentally-based approach, coupled with simulation models and other computational tools, allows the process engineer to determine the rate-limiting process(es) and analyze the potential for in situ bioremediation in the field.

While the experimental protocols for bench-, pilot- and field-scale treatability are rapidly emerging, there exist very few case studies in which predicted rates are actually compared with observed rates in the field. This lack of comparison with laboratory predictions of bioremediation processes with subsequent field observations is the largest single impediment to process engineering methodology development.

9. Conclusions

Many scale-dependent biotic and abiotic phenomena potentially limit the rate at which biotransformation can proceed in the field. Kinetic rates of reaction,
adsorption/desorption, interphase transfer, advective and dispersive transport, and field heterogeneities each may control bioremediation rates. Controlled study of these phenomena as they relate to bioremediation often requires isolation from the potential effects of other variables, which may compromise the usefulness of the results for scale-up. Very little has been reported wherein phenomena at more than one scale have been evaluated. This indicates the need for a process engineering approach to site remediation which integrates information from each scale of observation to formulate predictions of the effectiveness of various remedial treatments. This approach also demands that, regardless of the remedial design chosen, field data are collected and systematically compared to laboratory and modeling results, to evaluate the success of the scale-up process.

Acknowledgements

The authors acknowledge the support of the National Science Foundation through Cooperative Agreement ECD-8907039 between the National Science Foundation and Montana State University and the Engineering Research Center Industrial Associates, especially Conoco Inc. Funding was also provided through the Hazardous Substance Research Center (Agreement-R-815709) for U.S. EPA regions 7 and 8 at Kansas State University.

References


