TREATABILITY AND SCALE-UP PROTOCOLS
FOR POLYNUCLEAR AROMATIC HYDROCARBON
BIOREMEDIATION OF MANUFACTURED
GAS PLANT SOILS

FINAL REPORT
VOLUME 1

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TREATABILITY AND SCALE-UP PROTOCOLS
FOR POLYNUCLEAR AROMATIC HYDROCARBON BIOREMEDIATION
OF MANUFACTURED GAS PLANT SOILS

FINAL REPORT
VOLUME I

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GRI Project Manager
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July 1991
This report describes activities to develop a framework to reliably scale-up and apply challenging bioremediation processes to polynuclear aromatic hydrocarbons in Manufactured Gas Plant (MGP) soils. It includes: a discussion of the accuracy needed for competitive application of bioremediation: a framework and examples for treatability and scale-up protocols for selection, design and application of these processes: both batch and continuous testing protocols for developing predictive rate data: and special predictive relationships that may be used in process selection/scale-up.

This work, coupled with subsequent work (as recommended) to develop an MGP soil desorption/diffusion protocol and new scale-up methods, and with subsequent scale-up testing should lead to the capability for improved selection of MGP sites for bioremediation and improved performance, success, and reliability of field applications. With this greater predictive reliability, bioremediation will be used more often in the field on the most favorable applications and its cost advantages over other remediation options will be realized.
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The Gas Research Institute (GRI) has embarked on a multi-year program to address the Management of Manufactured Gas Plant (MGP) Sites. The program focuses on three primary technical areas: site investigation, risk assessment, and site restoration. Program activities in each of these areas are separated into two phases. Phase I emphasizes the identification, collation, and analysis of the state-of-the-art applicable to specific MGP site situations. Phase II involves the generation of data through targeted research and development projects.

The first phase of the program has produced a four-volume report which documents the state-of-the-art findings. The second phase of the program is producing a series of topical and final reports, such as this one, from laboratory and field experimental test programs. These programs are designed to fill data gaps required for the effective management of these sites and to advance the state-of-the-art for technologies determined during the first phase as potentially applicable to MGP site wastes. Other reports from the second phase will continue to be produced through 1991 as various portions of the program are completed.

This project, one of the Phase II efforts, has provided two final technical reports. Volume I deals with the results of this project from an applications engineering perspective. Volume II provides the scientific backup and support underpinning Volume I as well as specific scientific findings of importance to the ultimate application of environmental biotechnology to MGP site remediation. Accordingly, two report styles, engineering and scientific, will be evident in the respective documents.

**Final Report. Volume I. Treatability and Scale-up Protocols for Polynuclear Aromatic Hydrocarbon Bioremediation of Manufactured Gas Plant Soils** provides a discussion of the design accuracy required in order to reliably scale-up MGP
soil bioremediation processes; a framework and examples for both treatability and scale-up protocols for selection, design and application of these processes; a predictive relationship between PAH biodegradation kinetics and site characteristics; a continuous, dynamic protocol for testing process effectiveness, stability and robustness and determination of biodegradation rate models and constants in disturbed, field-like test systems; and a statistical analysis of the importance of analytical and experimental variation and error on reliable scale-up. Detailed experimental descriptions are available in an appendix under separate cover.

Final Report, Volume II. Molecular Bioanalytical Methods for Monitoring Polynuclear Aromatic Hydrocarbon Biodegradation in Manufactured Gas Plant Soils includes technical reviews of PAH bacterial metabolism, bioanalysis of PAH-degrading bacteria and their activity; an analysis of PAH-degrading activity in MGP soils; a discussion of a PAH-degrader culture collection developed during the project; and an analysis of the use of gene probes and other new approaches for improved bioanalysis of PAH degradation in MGP soil wastes.
RESEARCH SUMMARY

TITLE
Treatability and Scale-up Protocols for Polynuclear Aromatic Hydrocarbon Bioremediation of Manufactured Gas Plant Soils

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PRINCIPAL INVESTIGATOR
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Final Report, Volume I

OBJECTIVE
To develop testing and evaluation protocols for polynuclear aromatic hydrocarbon (PAH) bioremediation processes that lead to improved reliability in the field-scale remediation of manufactured gas plant (MGP) site materials.

TECHNICAL PERSPECTIVE
The management of manufactured gas plant sites may have to be addressed as part of voluntary property redevelopment actions or evolving regulatory requirements. The nature of many chemicals-of-interest present in the soils at these sites make them candidates for biological treatment. This management strategy represents a potentially technically viable alternative that may be capable of cost-effectively treating the soils and reducing future environmental liability associated with these residues.
While prior studies have suggested that many of the compounds present in these residues are biodegradable, prediction of the biokinetic rates needed for field-scale design and performance estimation of engineered bioremediation processes may be difficult. The highly concentrated, often tarry and viscous nature of MGP soils, the variable but often long-term weathering of MGP soils, and the often variable nature of the fundamental biodegradation processes lead to a unique and challenging application for bioremediation.

Exploitation of the potential of MGP site bioremediation at the commercial scale will require the ability: 1) to select the most appropriate and predictable sites on which to use bioremediation while rejecting those sites where performance is either poor or uncertain and 2) to develop an accurate prediction of the field-scale performance of a given design. According to an informal consensus of bioremediation professionals, application of bioremediation processes will require relative accuracy in the prediction of field-scale costs and performance (treatment times and final removal effectiveness) of around ±20% if the technology is to compete successfully with other remediation alternatives.

This requirement establishes the criterion for accuracy and precision needed for bioremediation testing and evaluation (treatability) and scale-up protocols. An improved approach over that currently used is needed for reliable PAH bioremediation technology application to MGP site materials.
RESULTS

An overall testing and scale-up protocol framework for PAH bioremediation processes on MGP sites is proposed that includes: 1) determination of PAH biotransformation and desorption/diffusion rates in separate experiments, 2) determination of idealized PAH biotransformation rates from both standardized batch and continuous test reactor systems, 3) a concept for quantification of desorption/diffusion rates in test systems, and 4) a concept for the development of "scale-up" relationships for future improved field performance prediction.

Naphthalene, phenanthrene, and anthracene batch idealized biotransformation kinetic potential (IBKP) tests were performed in small batch vials each containing one of five MGP soils. A radiolabeled PAH was used and led to improved analytical precision as compared to classical PAH chemical analysis. This improved analytical precision enabled an analysis of the complex nature of the biotransformation rate mechanism with the conclusion that batch PAH biotransformation in MGP soils is generally non-elementary (e.g., not zero-, or first-order) and may vary with time over the experiment (time-variant). These results indicate that conventional bioprocess modelling approaches to design and scale-up of batch bioremediation processes may be inherently less certain than those for non-biological remediation processes.

The improved analytical precision and the use of a pseudo-first-order biotransformation rate equation based only on the initial and final PAH concentrations led to a statistically significant relationship (correlation coefficient, $r^2 = 0.978$) between phenanthrene biotransformation rate constants in
these standardized batch IBKP tests based only on the initial total PAH concentration. This indicates that creation of meaningful and reliable scale-up relationships to improve early selection of the best (and early rejection of the worst) cases of MGP soils for bioremediation may be possible.

A continuous IBKP test of naphthalene biotransformation in a dynamically-perturbed, continuous soil slurry reactor charged with an MGP soil was developed to: 1) examine whether batch and continuous kinetic results can be compared, 2) to determine what impact dynamic field-scale disturbances might have on PAH biotransformation kinetics, 3) to estimate short-term PAH desorption/diffusion behavior in a continuous system operating on MGP soils, and 4) to test the feasibility of extracting process models ultimately for design and scale-up from experimental, time-series input-output data.

While first-order rate behavior varied widely between the batch and continuous IBKPs, comparison of the second-order rates between different lab-scale systems was encouraging. The batch IBKP second-order rate behavior may offer an estimate of the worst-case second-order rate constant for the continuous system and the first step in a reliable scale-up procedure.

Continuous IBKP test results for the MGP soil tested suggest a system prone to time-variant state changes (often driving the naphthalene concentrations to undetectable levels, but otherwise a stable and robust naphthalene-degradation process. Rates of naphthalene biotransforma-
tion were resistant to temperature and concentration changes and were adversely affected only when oxygen availability was severely restricted.

Two types of sorption/desorption behavior for PAHs in MGP soils were identified based only on the dynamic behavior. "Fast" sorption/desorption processes (less than 24 hours in response time) could be quantitatively analyzed in the continuous IBKP experiments. "Fast" naphthalene sorption/desorption rates in high organic carbon MGP soil were indistinguishable from those seen in uncontaminated control soils. "Slow" sorption/desorption processes were documented in special experiments and led to the hypothesis that diffusion within the soil tar phase observed sometimes may be the rate-limiting step for biotransformation of PAHs on MGP soils.

Batch IBKP results using conventional analysis (including extraction and analysis of the soil phase) likely include the effect of the "slow" processes, where neither the batch IBKP using labeled analysis nor the continuous IBKP "see" the slow effects. For these reasons, batch labeled and continuous IBKPs tend to measure only biotransformation and minimize "slow" sorption/desorption effects and may lead to valid comparisons of biotic rates.

The continuous IBKP Rate Equation was found to be first-order using parameter estimation techniques, as opposed to the non-elementary, time-variant rate behavior found for batch processes. No phenomenological process model based on frequency response was needed for this MGP soil slurry system.
Task 3. Development of a Calibration/Scaling Factor Protocol: To develop a calibration/scaling factor protocol to relate biotransformation in one lab-, pilot-, or field-scale test with other such tests.

Task 4. Application of Combined Protocols to Town-Gas Sites: The application of the above tools and protocols to one or more actual GRI town-gas sites with a process design and forecast of the system performance, and

Task 5. Verification of the Predictive Protocols: The verification of the predictive and scaling capability through support of efforts by other GRI contractors to implement environmental biotechnology at pilot and field scales.

Parallel treatability study activity was implemented by other GRI contractors (ReTeC, Inc.) on a variety of MGP site materials early in the overall GRI program and difficulty in selecting a site for field-scale application was encountered leading to a focus on the Task 1 and 2 objectives throughout this project. Task 3 was, with the concurrence of GRI project management, postponed to later projects. Task 4 became a cooperative effort across GRI contractors to test and analyze a number of MGP wastes and became a varied source of materials from which to recover organisms and determine kinetic activity for both Tasks 1 and 2. Task 5 was postponed to a later effort.

PROJECT IMPLICATIONS

This work, coupled with subsequent work to develop a MGP soil desorption/diffusion protocol and scale-up protocols, and subsequent scale-up testing should lead to the capability for improved selection of MGP sites for bioremediation.
Continuous bioremediation processes had higher biotransformation rates versus batch processes and may have advantages in both performance and stability as compared to batch processes. These features may make continuous processes (continuous in terms of liquid feedstream) attractive even though higher capital and operating costs may occur expected.

These lab-scale methods are not expected to provide rate information directly applicable to field-scale systems. Rather, new "scale-up" relationships should be developed as both standardized lab-scale and field-scale rate data are developed. Because of delays in planned field-scale trials, these "scale-up" relationships could not be developed as initially planned in this work.

This project was initially structured as a number of Tasks together accomplishing the overall objective of development and verification of an improved bioremediation testing and evaluation protocol.

Task 1. Development of New Molecular Tools to Enumerate Degradative Organisms: To develop molecular tools from degradative genes to track microorganisms capable of degrading polynuclear aromatic hydrocarbons and other compounds of interest in town-gas sites,

Task 2. Development of a Dynamic Systems Analysis Protocol: To develop a protocol for dynamic systems analysis of the biological system,
and improvement in performance, chances for success, and reliability of cost estimates. With this greater predictive reliability, bioremediation will be used more often in the field on the most favorable applications and its cost advantages over other remediation options will be realized by the Gas Industry.

Because of delays in field applications planned for other components of the overall GRI program, these methods have not been tested to establish whether they actually improve scale-up reliability. These and possibly other treatability and scale-up protocols should be incorporated into future pilot- and field-scale bioremediation tests to determine the potential for improved reliability for MGP PAH bioremediation applications.

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1.0 TREATABILITY AND SCALE-UP BIOREMEDIATION PROTOCOLS

1.1 PREDICTIVE RELIABILITY IN BIOREMEDIATION

Throughout this decade as bioremediation has emerged and been tested as a potentially economical waste treatment alternative, difficulties in predicting or maintaining the predicted field-scale performance of these processes have from time-to-time been experienced. Questions relating to the nature of appropriate biological testing of hazardous wastes to determine field-performance parameters have been posed:

1. Compound fates established in lab studies may be substantially different than in pilot-, full-, or even other lab-scale studies; 2. Positive biotransformation results found in small-scale lab systems are often not reproduced in different systems or even in the same systems at different times; 3. Where a given specific compound biotransformation is achieved at a larger scale, the kinetics are often unrelated to distributed measures of biomass; 4. Instantaneous biotransformation rates vary widely and in an apparently stochastic manner even in "well-operated, steady-state" systems; 5. Effects of competing fate mechanisms such as volatilization/stripping, sorption, and chemical reactions are not well understood at any scale and reported biological removal of a compound often includes removal/conversion related to competing abiotic processes. Comparisons of kinetics across systems and waste streams are confounded; 6. Competing abiotic mechanisms may be quite configuration-specific and will likely scale differently than the biotransformation mechanism.

For owners of problematic MGP waste sites to accept bioremediation as a treatment alternative, it is critical for the users of the technology (engineering firms, environmental biotechnology vendor companies, etc.) to be able to predict the field-scale performance of bioremediation from the behavior of various site samples with at least the same confidence as other technical treatment alternatives can be predicted and scaled. This requirement is in addition to the normal process selection requirements of process-specific effectiveness, process economy, safety, and so on.
At a recent symposium on the topic of several professionals in the bioremediation field were informally questioned about the acceptable error they believed sustainable in field-scale cost and performance predictions without putting them either at economic risk or impairing their competitiveness. An informal consensus was that accuracy in cost estimates of about ±20% was needed. For an in-situ or land-farming batch process, cost is roughly proportional to treatment time, therefore design treatment time should be accurate to about ±20% (e.g., 5 months give or take one month).

A paucity of reported information and major confidentiality concerns about field-scale predictive reliability complicates the assessment of whether the ±20% target is currently being achieved. However, imprecision in one factor known to contribute to inaccurate estimates is easy to document—chemical analysis in complex matrices. Recent studies with a number of MGP soils² demonstrate that traditional PAH chemical analysis on replicates of the soils can often vary 100-200% of the mean of the replicates. Likewise, others have reported coefficients of variation (the standard deviation divided by the mean) for biological soil treatment of coal gasification wastes for the PAH compounds naphthalene, acenaphthene, phenanthrene, and benz(a)anthracene of between 37-69% and 23-157% for the initial and final soil concentrations, respectively.³ While this effect has been attributed to non-homogeneous sampling difficulties, these variations directly affect the confidence intervals on rate parameters derived from these data. This will be discussed further in § 2.4.2.

The importance of the issue of improved predictive reliability for bioremediation testing is evidenced by a recent draft treatability protocol proposal circulated by the EPA. This proposal recognizes the need for national standardization of a tiered treatability protocol approach beginning with a laboratory screening evaluation:⁴

² Environmental Biotechnology—Moving From the Flask to the Field, Symposium, University of Tennessee, Knoxville, J. W. Blackburn—Symposium Coordinator, October, 1990.

³

⁴
• Laboratory screening is the first level of testing. It is used to establish the validity of a technology to treat a waste. These studies are generally low cost (e.g., $10K-50K) and usually require hours to days to complete. They yield data that can be used as indicators of a technology’s potential to meet performance goals and can identify operating standards for investigation during bench- or pilot-scale testing. They generate little, if any, design or cost data and should not be used as the sole basis for selection of a remedy.

• Bench-scale testing is the second level of testing. It is used to identify the technology’s performance on a waste-specific basis for an operable unit. These studies generally are of moderate cost (e.g. $50K-250K) and may require days to weeks to complete. They yield data that verify that the technology can meet expected cleanup goals and can provide information in support of the detailed analysis of the alternative...

• Pilot-scale testing is the third level of testing. It is used to provide quantitative performance, cost, and design information for remediating an operable unit. This level of testing also can produce data required to optimize performance. These studies are of moderate to high cost ($250K-1,000K) and may require weeks to months to complete. They yield data that verify performance to a higher degree than the bench-scale and provide detailed design information. They are most often performed during the remedy implementation phase of a site cleanup, although this level may be appropriate to support the remedy evaluation of innovative technologies.

This kind of treatability scale-up approach is a standard engineering procedure long known and used in many engineering disciplines in order to scale-up physical, chemical and biological processes. Central to the application of this type of approach is the premise that changing the size or physical dimensions of the test produces changes in biological system structure requiring adjustments of equations or parameters in the design model. These might range from different expressions for the kinetic Rate Equations to simple modifications of, say, one or more mass transfer constants. The need for scale-up experiments, equations or protocols arises from our inability to describe and solve the complete set of descriptive differential equations—often the hydrodynamic flow, the equations describing ecological interactions, or the equations describing important cellular metabolic processes.5

A common assumption has been that the system structure remains essentially the same over time. In other words, the design model applies a
priori to the field-scale case and is time-invariant. Unfortunately, for mixed-culture biological systems of importance in environmental processes, these assumptions—so well accepted in the application of physical, chemical or thermal environmental processes—may not generally apply to biological treatment systems. In an inherently changing system, the populations and processes are in a state of flux and the system’s biodegradation activity may vary significantly from moment-to-moment in either a well-operated reactor system or a natural "environmental reactor" (e.g. land treatment, in-situ, etc.).

1.1.1 DESIGN EQUATIONS AND RATE EQUATIONS

One of the realities for designers of any environmental treatment process including environmental biotechnical processes is the necessity for predicting the performance of the process in terms of the level of treatment over a given time frame. This is needed in order to design the system, but is also essential in estimating the costs of treatment.

Equations that describe the rate of conversion of a compound to a product or intermediate are called Rate Equations and must be known in order to quantitatively describe and scale-up the conversion process. Generally, these equations are based upon elementary rate models that often describe chemical reactions. Elementary rate models are based on elementary reactions where the Rate Equation is suggested by a stoichiometric chemical equation which represents the actual mode of action.

Selection of a rate model and equation in the absence of published studies that report the form of the rate model (in chemical processes, libraries of literature exist reporting the nature of the rate models for thousands of chemical processes) must follow the procedure of taking chemical conversion data in a reactor of a known configuration and then fitting the experimental time-concentration profiles to elementary rate models until a good fit is achieved. If in a batch experiment, this happens to yield a straight-line fit of say:
...then the conversion reaction is said to be first-order in concentration. Similarly, the reaction can be zero-, second- or even fractional order with only minor variations in the data analysis. From now on in this report, the term "second-order" Rate Equation means a rate model first-order in both compound and biomass concentration. Formally, this type of rate model is known as "second-order overall."

When there is no direct correspondence between stoichiometric equations and the rate expression, we have a non-elementary reaction [and Rate Equation]....Nonelementary reactions are explained by assuming that what we observe as a single reaction is in reality the overall effect of a sequence of elementary reactions. The reason for observing only a single reaction rather than two or more elementary reactions is that the amount of intermediates formed is negligibly small and unmeasurable.

So even in chemical reaction analysis, it is often found that elementary rate models do not adequately describe the conversion of a given compound. Non-elementary rate behavior may be related to a network of reaction steps made of elementary models in series and parallel, time lags in the metabolic processes related to storage or absence of critical reactants, products or regulatory compounds, or to other types of nonlinearities or discontinuities in the process. Obviously, in these cases, the ability to extract rate models diminishes with the ability to analyze metabolic intermediates and to decode the complexities of the process mechanism and results in aggregation of mechanisms into more simplified compartments.

In the ultimate case where we haven't the experimental tools to understand the mechanism (a common event in chemical catalytic processes, for example) then a purely empirical equation must be used for the rate equation. The sub-disciplines of systems analysis and process control have
developed mathematical strategies for fitting a process response to an input using only the measured response to a measured input with the conversion process model remaining a "black-box." This approach may be applied to bioremediation systems and is discussed in § 3.0.

Once designers have a Rate Equation that describes compound conversion rates, it is incorporated into a Design Equation permitting the calculation of time-concentration information based on the given reactor type—batch, continuous or one of many possible variations. Design Equations are fundamentally derived from material balances specific to the actual system design using the following general approach:

\[
\text{Material Entering the System - Material Removed From the System = Rate of Accumulation or Depletion of Material in the System}
\]

Examples of Rate Equations for zero- and first-order rate models and the related Design Equations for batch and continuous (backmix) reactors are presented Equations A.1-A.12 (Appendix A). It is noted that more complicated forms may be required based upon the complexity of the rate mechanisms as well as more complex and nonideal reactor configurations. Many such cases have been analyzed in the chemical reaction engineering design literature.

The design and scale-up process now seems deceptively straightforward: first a Rate Equation and its related constants are selected from the literature or are derived from an experiment and subsequent curve fitting, and then this equation is incorporated into the appropriate Design Equation, and mathematically manipulated to provide a relationship between the feed concentration (continuous case) or initial concentration (batch case) of the system and the effluent concentration (continuous) or time-concentration profile (batch) of the system.
A number of critical problems increase the error and reduce the predictive reliability of this generally correct design and scale-up approach as it is applied to the problem of scaling-up environmental biotechnical processes. These include:

- The selection of the performance parameter to be predicted.
- The limited knowledge base on Rate Equations for environmental biotechnical processes.
- The general inadequacy of elementary rate models for describing *in-vivo* metabolic activity.
- The extreme experimental difficulties in establishing mechanistic metabolic knowledge in environmentally-operating systems.
- The reality that, unlike most chemical processes, microbial processes adapt in response to environmental changes leading to Rate Equations that vary in structure with time (time-variance).
- The wide variability in experimental test kinetic results, even from the replicate tests, complicating analysis, comparison and design.
- The ideal system Design Equations rarely exactly describe actual behavior in real engineered or natural biotechnical reactors (including in-situ reaction zones) and generally empirically-based scale-up predictive equations relating controlled system behavior to environmental operation must be employed.
- These scale-up predictive equations do not currently exist. Scale-up is currently performed based on heuristics and/or past experience.
1.1.2 PERFORMANCE PARAMETER SELECTION

In the past, the selection of a compound or parameter for determining system performance was based on the compound or parameter on which the system was regulated. Removal of non-specific parameters such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Organic Carbon (TOC) provided a convenient way to describe system performance—large removals of these non-specific parameters were recognized to result in an improvement in effluent quality. Further, while there was generally a large scatter in experimental data leading to the selection of a Rate Equation, it seemed that a given elementary rate model probably served as well as another, and the design process was thought to be insensitive to the formulation of the Rate Equation.

However, in today's world of concern about specific toxic compounds, system performance is increasingly specified in terms of removal of a specific compound. The Rate Equation for the specific compound's conversion can be estimated by a Rate Equation for a non-specific parameter if and only if the conversion rate behavior of the specific compound and the non-specific parameter are similar.

The probability of the conversion behavior of specific and non-specific parameters not being similar can be illustrated by looking at a simplified catabolic (degradative) biochemical pathway for toluene (Figure 1). Toluene is known to biotransform either to benzyl alcohol or to methyl catechol, depending on the bacterial organisms and genetics present and active in a given system. Oxygen is incorporated into the initial conversion and into later biotransformations at various points in the pathway. It is clear that measurements of the overall oxygen uptake of a microbial process with toluene present are averages taken over all of the specific oxygen-consuming biotransformation steps.
The same is true for the evolution of carbon dioxide from the catabolism of a given compound (biochemical breaking-down of an organic compound). Denoted carbon mineralization, the overall rate of evolution of carbon dioxide from an organic parent compound is a weighted average of the individual rates of specific catabolic steps.

Is the measured average oxygen uptake rate or mineralization rate useful in predicting the system's rate of conversion of toluene and therefore meaningful in a Rate Equation to predict toluene biotransformation? It is tempting to answer in the affirmative since non-specific parameters are much easier and less expensive to measure.

In any real system, the overall removal of toluene is described not only by biotransformation but by actions of abiotic fate mechanisms such as the stripping of toluene from the aqueous reactor liquid into the air in contact with the system. As shown in Figure 2, the biotransformation of toluene to either benzyl alcohol or methyl catechol changes the physical properties (Henry's Law constant and solubility) of the compound dramatically. Benzyl alcohol can be expected to strip at a rate about 10,000 times lower than toluene in a given system and would exhibit an approximately 100-fold higher solubility (therefore lower octanol-water partition coefficient). Prediction of the toluene stripping from an operating system is an essential part of the scale-up process.
Figure 1. A Simplified Biochemical Pathway for the Microbial Conversion of Toluene

Stripping and biodegradation in an operating system are coupled, each reducing the liquid concentration. In turn, the rate of each is usually related to the liquid concentration. The necessary biodegradation rate equation required to couple with the stripping rate is the one describing the biotransformation of toluene to benzyl alcohol or methyl catechol. Any other describes a different reaction or group of reactions and does not describe the biological removal of toluene.
Some have suggested that the rate of oxygen uptake in the first biotransformation of aromatic organic compounds may be the slowest biochemical oxygen uptake process in a pathway and may be rate-limiting. If so, and if an experiment can be designed to measure the initial oxygen uptake corresponding only to the first biotransformation step, some suggest that proportional oxygen uptake rate data may be collected.

Our work indicates that the biotransformation rate is often much faster than the mineralization rate—mineralization rates are often of the same order as the oxygen uptake rates. Even when initial oxygen uptake biochemical metabolic transformation is rate-limiting, the overall oxygen uptake will be weighted by the other oxygen uptake biochemical metabolic transformations under way in the cells and may quantitatively vary widely from the overall rate measured. Designing a suitable experiment may be difficult.

In general, the parameter required for scale-up prediction of a system's performance is the biotransformation rate of the parent compound to the first metabolite. In practice, this is arrived at by measuring the overall removal of the parent compound from the system and then correcting this for abiotic processes either by measuring them or by estimating them with models verified for that test system.

A systems analysis/identification strategy sometimes called parameter estimation can be used to calculate biotransformation rates or constants from a series of concentration and flow measurements. Parameter estimation will be discussed later (§ 3.1) in conjunction with dynamic analysis in a continuous PAH-degrading system.
Figure 2. The Impact of Biotransformation on the Physical Properties and Fates of Toluene Metabolites

Most data in the literature on microbial processes either make use of non-specific parameter kinetics or do not correct parent compound conversion data for abiotic processes arising in a given test method. Unfortunately, while it appears that the literature is rich with kinetic data, most of the reports have little utility for system scale-up and may even lead to greatly erroneous scale-up performance predictions with no warning prior to field-scale failure to meet predicted performance. With current knowledge, treatability and scale-up experiments are needed for each application of environmental biotechnology to PAH bioremediation in MGP soils.
1.1.3 COMPLEXITIES OF CATABOLIC RATE BEHAVIOR

In general, it is optimistic to expect elementary rate models to describe catabolic (biodegradation) processes. While a given biotransformation may follow elementary behavior, at least in the axenic (pure culture) or cell-free systems used by the microbiologists and biochemists, this conversion may be under the influence of a wide variety of biochemical and molecular "control loops" in the environmental system. Also, time lags and dead time between enzymatic conversion steps can affect the apparent biotransformation rates observed in the experiment. Networks of biotransformations can lead to dynamic and oscillatory behavior beyond description with simple models. In this context, the rate model is an approximation of observed behavior. The prefix of "pseudo-" is often affixed to such an approximation (e.g., pseudo-first-order model).

A characteristic of mathematical models of non-elementary and non-linear rate processes and their networks is the potential for such processes to possess more than one "equilibrium" operating state. The mathematical term for this effect is "bifurcation." Allowed to come to equilibrium, the system can settle into one of several states, and when perturbed by outside influences can shift to another operating state. It is possible, that a system steadily biotransforming, say toluene could receive an upset in some environmental condition that shifts either the toluene-degrading organisms or the toluene-degrading pathway to another state with different biotransformation kinetic behavior. This a type of system time-variance.

Extrapolating rate behavior from systems operated in a typical laboratory steady-state mode can be dangerous if the system does not happen to be robust (resisting changes in activity in the face of environmental disturbances that the field-scale system may experience at the field scale). Dynamic response testing may be required if the tendency for upsets in biotransformation rates is important. Dynamic microbial system analysis of biological treatment systems at the laboratory scale was first demonstrated on
environmental biotechnical processes by Blackburn 9, 10—a PAH-degrading activated sludge system. Strong evidence that perturbations of naphthalene fed to this continuous system led to a significant change in naphthalene biotransformation response was reported.

1.1.4 SCALE-UP OF PERFORMANCE DATA FROM TEST SYSTEMS

Engineering Design Equations for all chemical or biochemical conversion processes (including MGP bioremediation systems) are based on ideal physical configurations that either represent or can be adjusted to represent behavior in engineered vessels. Reactor designers have needed to scale up from smaller-scale system results for decades and have relied on the development of "Scale-up" Equations to provide for estimates of rate behavior at different configurations, sizes or operating conditions than the test. As an example, transfer rates of oxygen between air and low viscosity liquids in agitated fermentation systems is given by Van't Riet11:

For a stirred vessel with water as the liquid and under coalescing agitation conditions, \((V \leq 2600 \text{ L}; 500 < P/V < 10,000 \text{ W/m}^3)\):

\[
k_{\alpha} = 2.6 \times 10^{-2} \left(\frac{P}{V}\right)^{0.4} (u_{gs})^{0.5}
\]

For a stirred vessel with water as the liquid and under noncoalescing agitation conditions, \((2 < V < 4400 \text{ L}; 500 < P/V < 10,000 \text{ W/m}^3)\):

\[
k_{\alpha} = 2.0 \times 10^{-3} \left(\frac{P}{V}\right)^{0.7} (u_{gs})^{0.2}
\]

In the above equations, \(u_{gs}\) represents the superficial gas velocity in the reactor in \(\text{sec}^{-1}\) and \(P/V\) is the power input per unit volume. Many such
equations are known for engineered systems and it is the responsibility of
the designer to carefully select and apply these scale-up predictive equa-
tions.

In principle, it should also be possible to develop similar "scale-up" pre-
dictors that adjust biotransformation kinetics from a standardized test experi-
ment to account for physical and configurational differences in a given field
application. Although the development of scale-up predictors require both
lab- and field-scale results from which the empirical equations can be
extracted (and no field-scale results were available to develop these equa-
tions in this work), future marriage between treatability protocols and field
tests should include scale-up predictor development as a priority.

1.2 OVERALL TREATABILITY AND SCALE-UP PROTOCOL CONCEPT

Treatability protocols for contaminated soils have been reported and
reviewed in another part of the GRI Program. In the same work, a treatability
protocol was proposed. This was called the GRI Accelerated Treatability
Protocol:

Given this picture of contaminant removal from soil systems, a crucial question arises: Does the historical treatability protocol, featuring soil pan or microcosm studies as its centerpiece, provide the information needed to evaluate the ability of a soil to remove contaminants by biological mechanisms and does it provide this information in a timely and cost-effective manner? Currently, soil pan or microcosm studies represent the best available laboratory models for simulating the expected performance of land treatment systems. However, these studies typically take four to six months to conduct and six to nine months to complete when time for chemical analysis, data interpretation, and report writing are included. In addition, soil pan studies provide a gross measure of contaminant removal, but do not provide information regarding the underlying processes influencing bioremediation of soils, such as information on partitioning of contaminants between soil solids and soil water.
The key parts of the GRI Accelerated Treatability Protocol are:

- **Soil Characterization**: The soil is characterized physically to determine the particle size distribution; chemically to determine concentrations of total organic carbon and chemicals-of-interest...; and microbially to determine the total microbial populations and the portion of this population capable of degrading PAH compounds. Characterization provides essential information on soil texture and contaminant loading.

- **Desorption Testing**: Equilibrium desorption of the chemicals-of-interest from the solid to the aqueous phase is determined, resulting in an estimate of the equilibrium partition coefficient, $K_p$. If the concentrations of chemicals-of-interest in the aqueous phase are high enough, then contaminant removal by biodegradation is likely. Estimates of $K_p$ are useful in assessing the transport and fate of chemicals-of-interest in the soil, as well as assessing the treatability of the soil.

- **Slurry Reactor Testing**: The potential treatment endpoint for the soil after biological processing is evaluated in [batch] slurry reactor testing. The [batch] slurry reactor provides an optimal environment for contaminant removal from soil because the contents are agitated, fostering transfer of chemicals from the soil to aqueous phase, and the reactor is highly aerated. Although rate information is provided by these tests, the primary emphasis is comparing concentration of chemicals-of-interest in the soil at the beginning and end of the study.  

The protocol's limitations were also noted:

While it is important to recognize the advantages of the GRI Accelerated Treatability Protocol, it is also important to recognize the limitations of this protocol and where significant knowledge gaps still exist. First, although rate data are provided by the [batch] slurry reactor, these data cannot be translated to field conditions at this time. Second, and more importantly, the assumption that the treatment endpoints obtained in a [batch] slurry reactor will mimic those that can be obtained in the field (or a soil pan experiment) remains a largely unverified supposition....

The GRI Accelerated Treatability Protocol, developed by ReTeC, Inc., has as its objectives 1) the inexpensive screening of MGP soils to identify the best options for field-scale testing and 2) to define the "endpoint" concentrations that can be achieved by batch bioremediation processes. The limitations
include the inability to scale the removal kinetics to reliably predict full-scale performance and uncertainty whether the experimental endpoint concentrations really match field-scale results.

In this report, an overall scheme for integrating the screening-type tests (like the GRI Accelerated Treatability Protocol) with predictive scale-up capability is proposed. Figure 3 presents a generalized schematic diagram of the network of relationships required for both treatability and scale-up predictions. The environment and process is generalized to represent tests and trials at all scales. To determine both kinetics and the impact of perturbations on the kinetics, the operating test or system is either monitored in time-series, or samples of "biomass" are taken periodically and assayed offline.

Like the Accelerated Protocol, separate, standardized tests are used to quantify both the desorption/diffusion behavior and the intrinsic biotransformation kinetic potential (IBKP). The IBKP is a measure of the biotransformation rate of the PAH available for catabolism in the aqueous reactor liquid or soil water. Unlike the Accelerated Protocol, emphasis is placed on the rates of desorption/diffusion and biotransformation potential with the hypothesis that four cases require evaluation for the selection of PAH bioremediation processes:

• Both desorption/diffusion and IBKP rates are greater than some minimum criterion leading to a recommendation for bioremediation.

• Desorption/diffusion rates are below and IBKP rates are above some minimum criterion leading to scale-up predictions based on the desorption/diffusion rates. Recommendation depends upon the performance prediction.
- Desorption/diffusion rates are above and IBKP rates are below some minimum criterion leading to scale-up predictions based on the IBKP rates. Recommendation depends upon the performance prediction.

- Both desorption/diffusion rates and IBKP rates are below some minimum criterion leading to coupled scale-up performance predictions and very possibly an early recommendation against bioremediation.

Also differing from the Accelerated Protocol, the batch IBKP test developed in this work makes use of radiolabeled PAH analysis for greater precision in the time-concentration profile and the continuous IBKP test is designed to be able to evaluate the impact of environmental perturbations on the PAH biotransformation kinetics as well as to enable the extraction of a type of process model directly from the perturbation-response data. These issues are discussed in § 2.4.2.
Figure 3. A Concept for Reliable Treatability and Scale-up Protocols for PAH Biotransformation in MGP Soils

Once the treatability protocol relationship is confirmed for use relating MGP soils, a scale-up predictor relating the rates in the standardized tests to a field-scale application can be developed and used to predict biotransformation performance in the specific field-scale case. The critical component of this phase of work is to make certain that the standardized treatability protocol is implemented at the field demonstration scale as well as at the lab-scale so that the empirical scale-up relationship can be extracted from the data.

Two biotransformation test procedures are reported in this work—one based on small, batch vials and another based on dynamically-perturbed con-
Continuous soil slurry reactor systems. The batch method is discussed in § 2.0 while the continuous and dynamic method will be discussed in § 3.0. Both approaches are idealized in several ways and are likely to reveal optimal intrinsic PAH biotransformation kinetic potential (IBKP) where the slow desorption/diffusion process of PAH into or from non-aqueous phases in the contaminated soil is experimentally minimized because of the way the test is run (Figure 4). These IBKP biotransformation kinetics may be related from MGP soil to MGP soil, but are expected to be fast relative to actual field-scale trials using land-farming, in situ approaches, bioreactor or other field configurations. Therefore, further desorption/diffusion and scale-up protocols are needed in order to reliably use the idealized laboratory-based information to estimate field-scale performance.

Figure 4. Concept for a Laboratory-Based Treatability Protocol for MGP Soils

Radiolabeled analytical approaches for the batch tests are superior to conventional compound analysis for MGP soils and possibly for other highly contaminated materials. This comparative fact will be discussed in § 2.4.2. The batch IBKP method is based on the use of a radiolabeled parent compound and the estimation of its biotransformation by measuring the label in a hexane-extract phase. The continuous IBKP method makes use of highly sensitive dedicated chemical analysis of the PAH in the aqueous liquid and does not require a radiolabel for routine analysis.
Neither the batch nor the continuous IBKP method includes effects of long-dynamic rate-limiting processes, such as the slow nonaqueous phase compartment PAH desorption/diffusion process. This biotransformation limitation can be determined with a lab-scale abiotic mass-transfer type test on a given type of MGP soil. The GRI Accelerated Treatability Protocol includes an abiotic test to determine the solid-liquid PAH partition coefficient. A variation of the GRI Accelerated Protocol to determine the kinetics of the slow component of the desorption/diffusion process is suggested:

- Select several organic test compounds where biotransformation in MGP soils is very slow or can be completely inhibited by some experimental means.
- Obtain labeled forms of these test compounds so that the added compound can be discriminated from like unlabeled indigenous compounds.
- Add these labeled compounds to the inhibited MGP soil of interest in a standardized fashion and allow to set over a standardized time frame.
- Add this preloaded soil sample to a well-mixed test system, possibly with an aqueous phase present and conduct time-series analysis for the test compound to determine the overall desorption/diffusion rate behavior.

A quite different approach to determine the kinetics of the fast component of the desorption/diffusion process is discussed later in § 3.2.3. This information is needed in order to employ parameter estimation of biotransformation rates in the continuous IBKP test.

The batch IBKP tests can be used to predict overall PAH biotransformation (first and last sampling points) but not of the actual time-concentration profile. As will be seen in a later section (§ 2.2), the actual batch
biotransformation rate profile data is often not first-order and can deviate either positively or negatively from the overall first-order rate model assumption. The use of time-series sampling and analysis techniques (a number of samples taken at known intervals throughout the test periods) will aid in characterization of these profiles. With attention to the potential causes of variable non-first-order rate behavior, time-series sampling can be expected to lead to better predictive understanding of the nature of a MGP soil's deviation from first-order and the resulting improvement in selecting time-variant Rate Equations.
2.0 A BATCH INTRINSIC BIOTRANSFORMATION KINETIC POTENTIAL TEST

Two basic types of laboratory-scale batch kinetic tests are discussed in this section—one for MGP soils and another for axenic (pure culture) organisms. In the first, 2 g samples of actual MGP site soils were placed into several 25 mL vials along with 1 mL of a buffer solution. To this mixture, a solution of $^{14}C$-labeled naphthalene, phenanthrene, or anthracene in acetone carrier was added and the mixture was incubated at 26°C and shaken. After a period of time, triplicates of the vials were harvested for analysis by addition of acid with collection of $^{14}CO_2$. A hexane/isopropanol solution was added to the soil to extract the parent compound along with non-polar metabolites while the polar metabolites partitioned into the aqueous/alcohol phase. Finally the remaining soil was air dried and oxidized in a thermal oxidizer to attempt to quantify non-extractable label. All liquid phases were counted for radioactive decay in a liquid scintillation counter. Further details of the experimental procedure can be found in experimental descriptions T1CS-08A, T1CS-08N, T1CS-08P, T1CS-27A, T1CS-27N, T1CS-27P, T1CS-31A, T1CS-31P, T1CS-31N, T1CS-51A, T1CS-51N, T1CS-51P, T1CS-97N, and T1CS-97P in Appendix B or in related references.12, 13

The organism axenic test is identical to the soil test except that a known axenic inoculum was added in place of the MGP soil sample. A characterization of the contaminated MGP soils for pH, total organic carbon, total PAHs and particle size distribution is provided in Table 1. These analyses were provided by ReTeC, Inc.2
Table 1. Characterization of the Contaminated MGP Soils Tested\(^a\)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Date Collected</th>
<th>pH</th>
<th>Total Organic Carbon (mg/kg)</th>
<th>Total PAHs (µg/kg)</th>
<th>Percent Silt</th>
<th>Percent Sands</th>
<th>Percent Gravel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil A</td>
<td>4/20/89</td>
<td>7.1</td>
<td>426,016</td>
<td>3,729,906</td>
<td>4</td>
<td>75</td>
<td>21</td>
</tr>
<tr>
<td>Soil B</td>
<td>3/27/89</td>
<td>8.1</td>
<td>5,951</td>
<td>233,193</td>
<td>2</td>
<td>70</td>
<td>28</td>
</tr>
<tr>
<td>Soil C</td>
<td>10/12/89</td>
<td>9.0</td>
<td>32,883</td>
<td>3,621,013</td>
<td>38</td>
<td>59</td>
<td>3</td>
</tr>
<tr>
<td>Soil D</td>
<td>unknown</td>
<td>8.0</td>
<td>65,118</td>
<td>334,316</td>
<td>17</td>
<td>70</td>
<td>12</td>
</tr>
<tr>
<td>Soil E</td>
<td>11/16/88</td>
<td>7.0</td>
<td>4,189</td>
<td>454,097</td>
<td>3</td>
<td>79</td>
<td>18</td>
</tr>
</tbody>
</table>

\(^a\) Ref. 2. Significant figures as reported.

\(^b\) Numerical designations used in other GRI reports.

2.1 RESULTS OF LABORATORY-SCALE BATCH IBKP EVALUATIONS

2.1.1 MICROBIOLOGICAL ANALYSES

An early hypothesis of this work was that biotransformation kinetic predictions could be improved if the rate model assumed was second-order, that is, first-order in PAH concentration and first-order in genotype concentration (the organisms present possessing the genes necessary for biotransformation of PAHs). Samples of MGP sites were analyzed for genotypes carrying the NAH7 plasmid as well as for other microbial assays. The methods used are reported elsewhere.\(^{12,13}\) Table 2 presents the results of these microbiological assays.
Table 2. Results of MGP Soil Microbiological Analyses

<table>
<thead>
<tr>
<th>Soil</th>
<th>Total Heterotrophic Bacteria (cfu/g MGP Soil)</th>
<th>nah Gene Probe Positive Bacteria (cfu/g MGP Soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil A (27)*</td>
<td>5.6±0.8x10⁶</td>
<td>1.3±0.4x10⁶</td>
</tr>
<tr>
<td>Soil B (8)</td>
<td>2.2±0.3x10⁶</td>
<td>5.9±0.8x10⁵</td>
</tr>
<tr>
<td>Soil C (97)</td>
<td>1.2±0.2x10⁸</td>
<td>3.3±0.5x10⁶</td>
</tr>
<tr>
<td>Soil D (51)</td>
<td>7.8±1.7x10⁶</td>
<td>2.1±0.5x10⁵</td>
</tr>
<tr>
<td>Soil E (31)</td>
<td>1.0±0.3x10⁷</td>
<td>&lt;2.0x10⁶</td>
</tr>
</tbody>
</table>

* Numerical designations used in some GRI reports.

2.1.2 PHENANTHRENE BIOTRANSFORMATION AND MINERALIZATION

Figures 5-7 present the kinetic experimental data from the batch kinetic tests for phenanthrene biotransformation and mineralization in Soils A-E. The abscissas on these figures are not always divided into equal time intervals between samples and therefore the overall shape of the time-concentration profiles on these figures has no meaning.

The $^{14}$C-phenanthrene in the Hexane Extract (confirmed by liquid chromatography) is taken as the concentration used in all biotransformation kinetic calculations. The Radiolabeled Carbon Dioxide recovered was used to calculate mineralization kinetics. The Water Extract was the aqueous phase left after the hexane:isopropanol extraction and was thought to contain water-soluble metabolites of the phenanthrene biotransformation. The soils after extraction were combusted in a thermal oxidizer and the resulting $^{14}$CO$_2$ was included in the Soil Immobilized Radiolabel Category. The sum of all radiolabel recovered was included in the Total Radiolabel Recovered Category.
Figure 5. Results from Radiolabeled Phenanthrene Batch MGP Soil Fate Tests, MGP Soils A and B
Figure 6. Results from Radiolabeled Phenanthrene Batch MGP Soil Fate Tests, MGP Soils C and D
All tests demonstrated significant biotransformation and mineralization except the one for Soil C (Figure 6, Panel A). Here, all indications supported the observation of no statistically-significant biological conversion of phenanthrene.

One concern regarding this protocol was that the radiolabeled compound was added to the experimental vials dissolved in small volumes of acetone. While the volume of acetone was less than 2 μL (approaching the lower limit of liquid that can be reproducibly manually handled), it was a pure organic liquid and was added to an experiment containing 2 mL of aqueous buffer. Thus, the amount of organic carbon available for biological uptake was a significant fraction of the dissolved organic carbon in the aqueous extract from the MGP soil. This level of added exogenous carbon might inhibit or somehow interfere with PAH biotransformation.
An additional experiment was performed where MGP soils A and B were tested using the batch IBKP protocol with the modification of varying amounts of acetone added as the sample carrier (0.5 - 10 µL) for $^{14}$C-naphthalene. Soil A was used as an example of high organic MGP soil and Soil B was used as an example of a low organic MGP soil. With three replicates of each test, the average coefficients of variations of $^{14}$CO$_2$ generated for Soil A and Soil B were 0.250 and 0.236, respectively. The results of this experiment, expressed as a percent of the lowest level of acetone added is presented in Table 3.

Table 3. Effect of Acetone Solvent Carrier on the Cumulative Mineralization Rate of $^{14}$C-Naphthalene in Batch IBKP Tests.

<table>
<thead>
<tr>
<th>Volume of Acetone Carrier Added</th>
<th>Elapsed Time (hr)</th>
<th>2.0 µL</th>
<th>5.0 µL</th>
<th>10.0 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGP Soil A--High Organic Soil</td>
<td>0</td>
<td>0.983</td>
<td>1.26</td>
<td>0.854</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.88</td>
<td>1.49</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.855</td>
<td>0.739</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.841</td>
<td>0.872</td>
<td>0.687</td>
</tr>
<tr>
<td>MGP Soil B--Low Organic Soil</td>
<td>0</td>
<td>n.a. *</td>
<td>3.67</td>
<td>0.713</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.07</td>
<td>0.834</td>
<td>0.759</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.11</td>
<td>0.832</td>
<td>0.900</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.970</td>
<td>0.930</td>
<td>0.963</td>
</tr>
</tbody>
</table>

* n.a.: not available.

The results of this experiment indicate that acetone carriers for the radiolabel might have an effect on the mineralization kinetics for the MGP soils with higher organic content. For these soils, the mineralization rates early in the test may be increased with moderate additions of acetone, and
possible inhibitory effect later in the experiment for higher additions of acetone. Generally negligible effects were seen in Soil B. Nearly all effects are small in comparison to the coefficients of variation in the analysis itself. Therefore, the acetone carrier addition is not expected to have a major influence on the results and conclusions presented in this report.

General advantages of radiolabel experiments include the ability to determine the fates of compounds that may already be present in the samples without analytical interference and the ability to provide a radiolabel material balance on the labeled element. Throughout our effort to perform batch kinetic soil experiments we have observed that in most cases, the ability to recover the label from MGP soils diminishes as the batch experiments proceed. This effect is demonstrated here in Soils A, B, and D as diminishing Total Radiolabel Recovered.

Possibilities for the variation in the $^{14}$C recovery was incomplete desorption/diffusion and/or combustion in the oxidizer. Additional controls were set-up to determine if acidification and/or hexane extraction somehow affected desorption/diffusion of the $^{14}$C-PAH from the soil. Four treatments were set-up with seven MGP soils (the original five, A-E, and two additional designated G and N):

- Samples were acidified, incubated, and oxidized.
- Samples were acidified, incubated, hexane-extracted, and oxidized.
- Samples were treated with azide, incubated, and oxidized.
- Samples were treated with azide, incubated, hexane-extracted, and oxidized.

Two g of soil were mixed with 1 mL of a minimal salts buffer in a 25 mL screw-capped poly-TFE-sealed mineralization vial as described previously. $^{14}$C-Anthracene (56,600 radioactive disintegrations per minute, dpm) was added to each soil. Samples were treated with 0.5 mL of 2 N H$_2$SO$_4$ or 0.1%
sodium azide (final concentration) to inhibit biotransformations. The hexane extraction procedure has been described previously. Thermal oxidation times were either 4 or 8 minutes.

There was little or no difference in $^{14}$C-recoveries for hexane extracted treatments. Black carbon deposits were visible after oxidation on soils A, D, G, and N implying that oxidation was not complete. Inhibiting the samples by 0.1% azide or by acidification did not affect $^{14}$C recoveries.

In non-hexane extracted samples, oxidation did not yield 100% recoveries. Increasing the oxidation time from 4 min to 8 min yielded higher $^{14}$C-recoveries in only two cases. These data indicate that the thermal oxidation combustion process for these samples is inefficient, possibly due to the carbonization of the organic carbon present during heating and the capture of the radiolabel in this carbonized matrix. Other possible losses of $^{14}$C include loss of $^{14}$CO$_2$ due to a saturated CO$_2$ trap, a poor seal or volatilization of the $^{14}$C to the vial headspace.

Based on the precision determined for the replicate analysis, the data generated on radiolabel biotransformation, carbon dioxide production and polar metabolites in the water extract are considered reliable. Development of a more suitable thermal oxidation method that permits the oxidation of the carbonized phase is needed in order to close the material balance on MGP-type soils.

For easy visual comparison of phenanthrene biodegradation from soil-to-soil, Figure 8 presents the rates of $^{14}$C removal from the hexane extract and generation as CO$_2$ for Soils A, B, D, and E, with the same scales on each axis. The data for removal from the hexane extract phase is assumed to be the result of biotransformation, while the generation of carbon dioxide is mineralization. The shapes of these profiles allow comparison of whether the conversion process is rapid or gradual from soil to soil. Also, the com-
parison of the biotransformation rate with the mineralization rate permits the assessment of whether there are metabolic products forming and accumulating in the process, since the overall rate of mineralization should be equal to the overall rate of biotransformation if no radiolabeled metabolites accumulate.

Equation A.19 can be used to calculate pseudo-first-order biotransformation rate constants from the hexane extract concentration data and Equation A.20 can likewise be used to calculate pseudo-first-order mineralization rate constants. These are presented in Figure 9 for Soils A-E, as well as for three non-soil axenic (pure) cultures of phenanthrene degraders. These are *Pseudomonas putida G7* (Panel F), DFC50, (Panel G), and DFC 49 (Panel H). *P. putida G7* is the strain harboring the NAH7 plasmid which is a genetic element coding for naphthalene degradation and is used as a gene probe for PAH degradation in this work. The other strains were isolated from MGP soils early in the study and have been found to degrade PAHs. Note that axes are not scaled for direct visual comparison from panel to panel, rather each panel is independently scaled to permit close examination of the rate behavior.

The effect of using the pseudo-first-order rate constant (Figure 9) instead of the rate itself (Figure 8) is to "normalize" the rate behavior to the hexane extract concentration present at the time that rate is measured. Thus, high pseudo-rate constants can exist late in the experiment when the hexane extract concentration is low, and where rates would be necessarily low because of limited reactant (parent compound) present. The pseudo-first-order rate constant is also a test of whether or not the biotransformation or mineralization process is actually first-order. If it is first-order, the rate constant trace should be a straight horizontal line in Figure 9. Departure from this is evidence that the conversion processes are not first-order and in these cases indeed, vary with time. Phenanthrene biotransformation in these batch tests is non-elementary and/or time-variant.
Figure 10 presents the time-concentration profile for phenanthrene biotransformation batch testing of MGP soils and axenic organisms. For simplicity of comparison, an overall first-order assumption is made and Equations A.19 and A.20 are used with the initial and final concentrations, a first-order Rate Equation results with behavior shown in Figure 10. Departure from first-order behavior is again highlighted in this figure.

While the complex rate behavior is not modelled well with first-order Rate Equations, interval pseudo-first-order rate constants can be used in a model that assumes that first-order behavior exists between each measured point. The time-concentration profile can then be calculated beginning with the initial concentration then recursively to the last. The use of this sort of a recursive predictor using pseudo-first-order rate constants calculated for each sampling interval (Equations A.14 and A.15) is shown (Figure 10) as
long dashes slightly offset from the actual concentration data. While capable of accurately reproducing the observed concentration profile, this predictive approach is limited by the need to calculate and store as much rate constant information as the original concentration information. In other words, this model offers no generalization potential and therefore no advantage over use of the concentration data alone.

Other strategies exist to model non-elementary or time-variant rate behavior. One approach might be to use a pattern search approach for groups of data that fit an elementary model over a given interval and sequence these segments together for an overall Rate Equation. The recursive approach could then be employed with the advantage of generalization. Another approach might be to handle the rate constants as parameter distributions, with time being the independent variable. These distributions could be modeled with a variety of distributions or functions (e.g., ramp, Poisson, Weibull, exponential, normal, etc.). A worthy topic for future work is to formalize an artificial intelligent system to analyze complex rate behavior and generate the "best fit" for experimental data for use as predictive Rate Equations.

Overall pseudo-first-order rate constants calculated from Equations

Nomenclature for kinetic rate constants varies widely in the literature. The sign of the rate constant depends upon the formulation of the material balance and the definition of production. Classical chemical rate kinetics usually assume rates for chemical production as positive and related rate constants as also positive. Removals can be shown as negative rate terms or as positive rate terms and negative rate constants. The equations presented in Appendix A show removals as negative rate terms and therefore rate constants, so calculated should be positive.

However, in this work, biotransformation rate constants, \( k^t \), are reported as negative values to discriminate them from mineralization rate constants, \( k^m \) (reported as positive values). It is hoped that the loss of consistency is less problematic to the reader than the increase in clarity from not confusing the two types of rate constants reported in this work. Further description of nomenclature used to designate rate constants may be found in Appendix A, § A.6.
A.19 and A.20 are presented in Panel A of Figure 11. Overall pseudo-second-order rate constants based on Total Heterotrophic Bacteria Plate Counts, $k_2^{th}$ and $k_2^{th}$, are presented in Panel B. Overall pseudo-second-order rate constants based on nah gene probe-positive bacteria concentration, $k_2^{sp}$ and $k_2^{sp}$, are presented in Panel C of Figure 11.

Both second-order rate constants calculated with total heterotrophic bacteria and gene probe technology are shown as points of reference to current research activity by other groups. It seems clear that similar but not identical response patterns in second-order rate constants appear between $k_2^{th}$ and $k_2^{sp}$. As noted in a later discussion, § 2.3, the best predictive relationships for the batch test systems make use of the rate constant derived from gene probe technology ($k_2^{sp}$) as a measure of active PAH-degrading biomass.

A generalized analysis of error propagation presented in a subsequent section (§ 2.4.1, Figure 20) may be used to estimate the typical range of error in the first-order rate constants presented in Figure 11. Error in these constants depends upon the error in four other parameters: the overall conversion of the parent compound, the error in the analytical measurements of the initial and final chemical concentrations, and the error in the measurement of time between the initial and final samples.

As seen in a later section (§ 2.4.2), a typical relative standard error in the radiolabel concentration measurements is around ±10% (although potentially much greater for conventional analyses). As noted in experimental description in Appendix B, parent compound conversions exceed 60% and are often greater than 90% in the tests. If relative standard error in the time measurement is conservatively estimated as ±10%, then the relative standard error in the pseudo-first-order rate constants (Figure 11, Panel A) should not exceed ±20% and may generally be closer to ±10%.
Figure 9. Interval-Based Pseudo-First-Order Rate Constants for Radiolabeled Phenanthrene Biotransformation and Mineralization in Batch MGP Soil and Axenic Organism Tests
Figure 10. Actual and Predicted Radiolabeled Phenanthrene Concentrations in the Hexane Extract from Batch MGP Soil and Axenic Organism Tests
Figure 11. Overall Pseudo-First- and Second-Order Radiolabeled Phenanthrene Rate Constants from Batch MGP Soil and Axenic Organism Tests

Second-order constants were calculated by dividing the first-order constant by the appropriate microbial concentration from Table 2. With relative standard errors of from ±15-30% and with the general rule of error propagation, "where multiplication or division is involved, the relative determinate errors are transmitted directly to the result," standard errors of the pseudo-second-order rate constants should range from ±15-30%. It is interesting to note that the major source of error in the second-order calcula-
tions are the errors associated with the microbial analyses and these can be reduced in the future with use of more than three replicate samples for microbial analysis (see § 2.4.2).

2.1.3 NAPHTHALENE BIOTRANSFORMATION AND MINERALIZATION

Naphthalene was tested in a similar manner as the phenanthrene tests discussed above. Fate profiles as shown for phenanthrene are not shown for naphthalene, however, generally similar behavior and analytical error were experienced in the naphthalene studies.

Equations A.19 and A.20 were used to calculate interval pseudo-first order biotransformation and mineralization rate constants. These are presented in Figure 12 for Soils A-E, as well as for five non-soil axenic (pure) cultures of phenanthrene degraders. These are *Pseudomonas putida* G7 (Panel F), DFC50, (Panel G), and DFC 49 (Panel H), HK44 (Panel I), IGT 305 (Panel J), and IGT 306 (Panel K). HK44 is a genetically engineered microorganism in which a bioluminescent gene cassette has been inserted into the lower pathway NAH7 naphthalene-degrading genes in such a way as to couple the degradation of naphthalene with the evolution of light.\(^\text{15}\) The IGT strains were isolated by investigators at the Institute of Gas Technology\(^\text{8}\) and were kindly provided for these studies. A more detailed discussion of the nature of the axenic organisms is presented elsewhere.\(^\text{13}\)

As in the case of phenanthrene, an overall first-order assumption is made and Equations A.19 and A.20 are used with the initial and final concentrations and a first-order Rate Equation results with behavior shown in Figure 13. Again departure from first-order behavior is highlighted in this figure.

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\(^{8}\) Shrivastava, V. J., Institute of Gas Technology, Chicago, Il.
Overall pseudo-first-order rate constants calculated from Equations A.19 and A.20 using initial and final concentrations are presented in Panel A of Figure 14. Pseudo-second-order rate constants based on Total Heterotrophic Bacterial Plate Counts, $k_{2}^{\text{h}}$ and $k_{2}^{\text{n}}$, are presented in Panel B. Pseudo-second-order rate constants based on nah gene probe-positive bacteria concentrations, $k_{2}^{p}$ and $k_{2}^{n}$, are presented in Panel C of Figure 14.

As in the case of phenanthrene, the naphthalene conversion processes are not generally first-order and are non-elementary and/or time-variant. Standard error of the first- and second-order rate constants range ±10-20%, and ±15-30%, respectively.
Figure 12. Interval-Based Pseudo-First-Order Rate Constants for Radiolabeled Naphthalene Biotransformation and Mineralization in Batch MGP Soil and Axenic Organism Tests
Figure 13. Actual and Predicted Radiolabeled Naphthalene Concentrations in the Hexane Extract from Batch MGP Soil and Axenic Organism Tests
2.1.4 ANTHRACENE BIOTRANSFORMATION AND MINERALIZATION

Anthracene was tested in Soils A, B, D and E with generally similar but slower results relative to phenanthrene. Figure 15 presents the interval
pseudo-first-order rate constants calculated for anthracene biotransformation and mineralization, while Figure 16 presents the overall pseudo-first- and second-order rate constants for anthracene biotransformation and mineralization.

As in the case of phenanthrene, the anthracene conversion processes are not generally first-order and are non-elementary and/or time-variant. Relative standard error of the first- and second-order rate constants range ±10-20%, and ±15-30%, respectively.

Figure 15. Interval-Based Pseudo-First-Order Rate Constants for Radiolabeled Anthracene Biotransformation and Mineralization in Batch MGP Soil and Axenic Organism Tests
Figure 16. Overall Pseudo-First- and Second-Order Radiolabeled Anthracene Rate Constants from Batch MGP Soil and Axenic Organism Tests
2.2 DISCUSSION OF BATCH IBKP RESULTS

2.2.1 BIOTRANSMFORMATION VS. MINERALIZATION KINETICS

The overall pseudo-first- and second-order rate constants for PAH biotransformation and mineralization are tabularly presented in Table 4.

Table 4. Summary of Overall Batch Pseudo-First- and Second-Order Rate Constants for PAH Biotransformation and Mineralization

<table>
<thead>
<tr>
<th>Overall Pseudo-Biotransformation Rate Constants</th>
<th>Overall Pseudo-Mineralization Rate Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material Tested</td>
<td>Naphthalene</td>
</tr>
<tr>
<td>Sol A</td>
<td>-1.66</td>
</tr>
<tr>
<td>Sol B</td>
<td>-1.58</td>
</tr>
<tr>
<td>Sol C</td>
<td>0.102</td>
</tr>
<tr>
<td>Sol D</td>
<td>-1.20</td>
</tr>
<tr>
<td>Sol E</td>
<td>-0.12</td>
</tr>
<tr>
<td>P. purpurea</td>
<td>-0.73</td>
</tr>
<tr>
<td>C7 27</td>
<td>-1.87</td>
</tr>
<tr>
<td>DFC50</td>
<td>-1.25</td>
</tr>
<tr>
<td>DFC48</td>
<td>-1.14</td>
</tr>
<tr>
<td>HK44</td>
<td>-1.50</td>
</tr>
<tr>
<td>ICT 305</td>
<td>-2.74</td>
</tr>
<tr>
<td>ICT 308</td>
<td>-1.29</td>
</tr>
</tbody>
</table>

*Rate calculations are presented, but are not statistically significant.
Relative standard errors for the first-order and second-order rate constants (except as noted) range ±10-20%, and ±15-30%, respectively.

The radiolabeled carbon being converted to carbon dioxide as a percent of the removal of the parent compound is presented in Table 5. A high percentage indicates that mineralization is a dominant fate of the parent compound over the duration of the test. A low percentage may indicate that a significant fraction of the radiolabeled carbon in the parent compound has a fate other than mineralization. These may include conversion to polar metabolites, immobilization into the soil or biomass, or volatilization losses in the test. The latter possibility is unlikely since the test was conducted in a sealed vial and the relative standard errors between replicates of all samples were generally low (±5-15%)

Table 5. PAH Carbon Mineralization Rate as a Percentage of the Biotransformation Rate

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Naphthalene</th>
<th>Phenanthrene</th>
<th>Anthracene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil A</td>
<td>17</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Soil B</td>
<td>13</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Soil C</td>
<td>15</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>Soil D</td>
<td>23</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Soil E</td>
<td>24</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>P. putida G7</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>DFC50</td>
<td>13</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>DFC49</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>HK44</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGT 305</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGT 306</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.2.2 AXENIC (PURE CULTURE) VS. MGP SOIL KINETICS

The comparative kinetic behavior of axenic PAH-degrading organisms and indigenous PAH-degrading organisms is interesting. Table 4 indicates that overall naphthalene and phenanthrene pseudo-first-order biotransformation and mineralization rate constants ($k_1^*$ or $k_1^+$) for axenic organisms are of the same order as the fastest of the indigenous MGP soil organisms.
However except for MGP Soil C and naphthalene degradation in Soil E, pseudo-second-order rate constants based on gene probes for the MGP indigenous organisms have a greater magnitude than those for the axenic organisms. This finding indicates that indigenous organisms in MGP soil tend to degrade PAHs with greater efficiency than axenic organisms in culture. This is additional evidence that pure-culture test kinetic results should not be used to project field-scale activity.

2.2.3 RELATIVE PAH BIOTRANSFORMATION KINETICS

Table 6 presents the rates of naphthalene and phenanthrene biotransformation and mineralization relative to those of anthracene. Since the microbial concentrations are the same for each soil, these ratios should be independent of rate constant order.

Table 6. Biotransformation and Mineralization Rates of Naphthalene, Phenanthrene Relative that of Anthracene in MGP Soils

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Biotransformation</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Naphthalene:</td>
<td>Phenanthrene:</td>
<td>Naphthalene:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anthracene</td>
<td>Anthracene</td>
<td>Phenanthrene</td>
<td></td>
</tr>
<tr>
<td>Soil A</td>
<td>18</td>
<td>1.6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Soil B</td>
<td>21</td>
<td>5.6</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Soil D</td>
<td>3.9</td>
<td>3.7</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Soil E</td>
<td>5.9</td>
<td>3.3</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Mineralization</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Naphthalene:</td>
<td>Phenanthrene:</td>
<td>Naphthalene:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anthracene</td>
<td>Anthracene</td>
<td>Phenanthrene</td>
<td></td>
</tr>
<tr>
<td>Soil A</td>
<td>31</td>
<td>2.2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Soil B</td>
<td>100</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Soil D</td>
<td>14</td>
<td>7.3</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Soil E</td>
<td>8.8</td>
<td>3.8</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>
The PAH biotransformation rates compared to the anthracene biotransformation rate ranges approximately 5-fold while the naphthalene:phenanthrene ratio ranges 10-fold for MGP soils. Mineralization ratios to anthracene range 11-fold and 5-fold for naphthalene and phenanthrene, respectively and 7-fold for naphthalene relative to phenanthrene.

Constant relative degradation rates between PAHs are not maintained from soil to soil. Significant error can result if biotransformation rates of one PAH are calculated as a simple proportionality to others in the absence of supporting experiments. This approach is not recommended as a predictive method.

2.3 PREDICTIVE KINETIC RELATIONSHIPS FOR COMPARISONS BETWEEN BATCH TESTS

Biotransformation rate constants assuming overall first-order rate behavior were calculated from the initial concentration (hexane-extract) and the final concentration in the experiment. As shown earlier in Figures 9, 10, 12, 13 and 15, the first-order assumption does not model the time-series behavior well. However, it may be used to exactly model any initial and final concentrations of the experiment (for that matter any rate model will perfectly model two data points) with the implied caution that prediction of intermediate data is not reliable.

Pseudo-second-order rate constants were calculated by dividing the pseudo-first-order rate constant by a variable representing the biomass present. The pseudo-second-order biotransformation rate constant using total heterotrophic bacterial plate counts as the measure of biomass is denoted by $k_2^{bh}$ while the related constant based on nah gene probe-positive bacterial concentrations is denoted $k_2^{bP}$. The biomass in each case is $B^h$ and $B^P$, respectively.
Exploration for exponential and power relationships between both the measured initial TOC and total PAHs of five MGP soils (Table 1) and the pseudo-first- and second-order rate constants was then performed.

Logarithmic transformations of the independent variables and linear regression with the dependent variable permitted investigation of exponential or logarithmic relationships of the following form:

\[ y(x) = \alpha \ln(x) \]

Similarly, linear regression of both independent and dependent variables was used to test for power function relationships.

\[ y(x) = c x^d \]

The regressions also provided an estimate of the quality of fit (correlation coefficient, \( r^2 \)). Data on both naphthalene and phenanthrene were tested but naphthalene results were consistently poorer than phenanthrene and are not presented. Table 7 presents some of the more significant regression results.
### Table 7. Regressions for Predictive Kinetic Relationships

<table>
<thead>
<tr>
<th>Number of Soils</th>
<th>Independent Variable</th>
<th>Dependent Variables</th>
<th>Correlation Coefficient, r^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>log TPAH</td>
<td></td>
<td>k_i</td>
</tr>
<tr>
<td>5</td>
<td>log TPAH</td>
<td></td>
<td>k_i</td>
</tr>
<tr>
<td>5</td>
<td>log TPAH</td>
<td></td>
<td>k_i</td>
</tr>
<tr>
<td>5</td>
<td>log TOC</td>
<td></td>
<td>k_i</td>
</tr>
<tr>
<td>5</td>
<td>log TOC</td>
<td></td>
<td>k_i</td>
</tr>
<tr>
<td>5</td>
<td>log TOC</td>
<td></td>
<td>k_i</td>
</tr>
</tbody>
</table>

An excellent relationship for the prediction of k_i as a function of log TPAH was developed with a correlation coefficient, r^2, of 0.978 (Figure 17). TPAH has units of μg/kg and k_i has units of day^-1. The standard error from the use of this relationship is comparable to the experimental standard error estimated for pseudo-first-order rate constants (± 10-20%).

![Figure 17. Predictive Relationship for Phenanthrene Overall Pseudo-First-Order Biotransformation Rate Constants in MGP Soils](image)
2.4 ANALYSIS OF ALLOWABLE ERROR FOR RELIABLE SCALE-UP

2.4.1 IMPACT OF EXPERIMENTAL ERRORS ON THE RATE CONSTANT

Any experiment with the purpose of providing rate information for scale-up includes experimental error in the measurements for concentrations and time. For a batch-type test, the time is the sampling time for the concentration samples. In a continuous-type test, the time is the reactor residence time (influenced by flow and volume changes). These errors propagate to the value calculated for the rate constant for any Rate Equation assumed. The impact of the error is, however mitigated by the mathematical rate constant calculation itself.

A discussion of this error propagation process is given in § A-2. Note that this source of error is independent of inaccuracies in the selection of the Rate Equation and that incorrect or unknown time-variant Rate Equations may contribute additional error to the calculated constants.

Figure 18 describes the impact of measurement error propagated to the calculated rate constant for a first-order Rate Equation in an ideal continuous stirred tank reactor. In this analysis, Equation A.12 was solved for $k_1$ and $\pm 4$, and 10% errors were added to concentration and residence time measurements. Relative standard error in the rate constant was calculated according to Equation A.13 for the cases listed in Table A.1 (Appendix A) and the average (bold solid lines), minimum (short dashed lines), and maximum (long dashed lines) are shown for each error assumption as a function of the parent compound conversion (Equation A.4).
Figure 18. Error Propagation to the Calculated First-Order Rate Constant From Measurement Errors in Concentration and Residence Time in Continuous Tests

For comparative purposes, the desired relative accuracy (±20%) in the kinetic rate constant is shown. As noted earlier, this was determined heuristically by discussion with several environmental biotechnology professionals.

Figure 18 shows that with ±10% relative standard measurement error, the relative standard error in the calculated rate constant is less than ±20% only for systems with greater than 75% conversion of the parent compound. Relative standard errors for the lower measurement error case (±4%) tolerate lower parent compound conversions in order to meet the desired accuracy in the rate constant calculation. Obviously, when the measurement errors are higher than ±10%, greater than 75% conversion is needed to achieve constants with the desired accuracy. All else being equal, tests with lower conversions result in greater error in the calculated rate constant.

A similar analysis may be made for zero- and first-order rate constants calculated from data in batch experiments using Equations A.5-9 (Figure 19).
In this analysis the relative standard errors are shown for first-order (solid line) and zero-order (dashed line) rate constants—the minimum and maximum values are omitted. The time measurement refers to the time between samples in the batch test.

![Graph showing error propagation to the calculated zero- and first-order rate constants from measurement errors in concentration and sampling time in batch tests.](image)

**Figure 19.** Error Propagation to the Calculated Zero- and First-Order Rate Constants From Measurement Errors in Concentration and Sampling Time in Batch Tests

Zero- and first-order Rate Equations propagate error in a similar fashion. Here too, relative measurement errors of ±10% require a parent compound conversion of nearly 60% to achieve the desired accuracy in the rate constant.

In summary, continuous systems where the conversion of the parent compound is low lead to rate constant calculations with inherently more error than systems where the conversion is high. Batch systems with relative standard measurement error greater than or equal to ±10% require a parent compound conversion of at least 60% in order to produce a calculated rate constant with average relative accuracy of ±20% or better. Batch-derived
kinetics with either conversions less than 60% or relative standard measurement errors greater than ±10% will result in calculated rate constants that on the average exceed the desired ±20% accuracy needed for reliable scale-up.

2.4.2 IMPACT OF CHEMICAL ANALYSIS ERRORS ON RATE CONSTANT ERROR

The measurement errors for concentration depend greatly on the compound measured, the method used, and the sample soil matrix itself. In analytical work on MGP soils by ReTeC\(^2\) (Table 8), coefficients of variation (CV, standard deviation divided by the mean) for the initial concentration of Total PAHs ranged from 0.24 to 1.08 for the soils discussed in this report. Naphthalene and phenanthrene CVs ranged from 0.41-1.34 and 0.78-1.48, respectively.

Table 8. Coefficient of Variation Error Statistic for Conventional Chemical PAH Analysis in Initial MGP Soils

<table>
<thead>
<tr>
<th>MGP Soil</th>
<th>Number of Replicates</th>
<th>Naphthalene CV*</th>
<th>Phenanthrene CV*</th>
<th>Total PAHs CV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil A</td>
<td>4</td>
<td>0.41</td>
<td>1.12</td>
<td>0.24</td>
</tr>
<tr>
<td>Soil B</td>
<td>7</td>
<td>0.67</td>
<td>1.34</td>
<td>0.64</td>
</tr>
<tr>
<td>Soil C</td>
<td>10</td>
<td>0.51</td>
<td>1.08</td>
<td>0.52</td>
</tr>
<tr>
<td>Soil D</td>
<td>11</td>
<td>0.65</td>
<td>0.78</td>
<td>0.25</td>
</tr>
<tr>
<td>Soil E</td>
<td>7</td>
<td>1.34</td>
<td>1.48</td>
<td>1.08</td>
</tr>
</tbody>
</table>

* CV = coefficient of variation = standard deviation/mean.

An inherent advantage of the radiolabeled method described in this work is that the CVs for radiolabeled analysis in the batch tests described earlier are significantly lower than actual chemical analysis, even in the same sample matrix (Table 9). Of the same magnitude as measurement errors...
assumed in Figures 18 and 19, the radiolabel approach appears to offer inherently greater accuracy in the resulting rate constant calculation than does conventional chemical analysis for PAHs in MGP soils.

Table 9. Coefficient of Variation Error Statistic for Radiolabeled PAH Analysis in Initial MGP Soils and Average for a Biotransformation Test, Three Replicates for Each Sample

<table>
<thead>
<tr>
<th>MGP Soil</th>
<th>Naphthalene CV*</th>
<th>Phenanthrene CV*</th>
<th>Anthracene CV*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Average</td>
<td>Initial</td>
</tr>
<tr>
<td>Soil A</td>
<td>0.01</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>Soil B</td>
<td>0.07</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>Soil C</td>
<td>0.04</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>Soil D</td>
<td>0.05</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Soil E</td>
<td>0.02</td>
<td>0.18</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* CV = coefficient of variation = standard deviation/mean.

With a normal distribution assumed for the rate constant (this will be discussed further), the accuracy also depends upon the number of kinetic tests performed. The relative error of the mean rate constant value can be related to a function of the coefficient of variation and the number of kinetic tests by manipulating the following equation:¹⁶

\[ n_x = \left( \frac{z_{0.05}}{\bar{x} - \mu} \right)^2 \sigma^2 \]

In this equation, the number of replicate tests is \( n_x \), \( z_{0.05} \) is a statistic based on the selection of a 95% confidence limit, \( \bar{x} \) is the mean of the replicate tests, \( \mu \) is the true population mean, and \( \sigma^2 \) is the variance of the replicate samples. Rearranging this equation in terms of relative percent error,
\[ \epsilon = \frac{z_{0.05}}{\sqrt{n}} \times 100 \]

Figure 20 presents this relationship to indicate the importance in increasing the number of replicate tests as the coefficient of variation of between tests increases.

Figure 20. Relationship Between the Number of Replicate Kinetic Tests, the Coefficient of Variation Between Rate Constants Between Replicates and the Relative Error of the Calculated Mean (95% Confidence Interval)

When the coefficient of variation between replicates tests is low, then the effect of increasing the number of replicate tests on the accuracy of the calculated rate constant is small. However, when the coefficient of variation
between replicate tests is high, more replicates are required to assure the same low relative error in the calculated rate constant. If it can be assumed that the coefficients of variation between calculated rate constants from replicate kinetic tests using conventional PAH chemical analysis will be greater than using radiolabeled PAH analysis (Tables 8 and 9), then using radiolabeled analysis should also reduce the number of experiments needed for the same accuracy in the calculated rate constant.

2.4.3 IMPACT OF RATE CONSTANT ERROR ON SCALE-UP

If a first-order Rate Equation is assumed, if the rate constant has been determined with known precision (expressed as a coefficient of variation) and if no additional scale-up predictive equation is needed, the time for treatment to a given level of parent compound conversion in a batch system can be found by solving Equation A.6 or A.8 for the treatment time, \( t \). Normalized to the conversion half-life:

\[
t_{1/2} = \frac{0.693}{k_1}
\]

...the ±95% confidence intervals and ± standard error interval in the resulting dimensionless time for treatment related to coefficients of variation of the rate constant, \( k_1 \) is presented in Figure 21 for the case where 90% of the parent compound is biotransformed.

The impact of error in the kinetic rate constant on the time of treatment and related economics is significant. As the coefficient of variation of \( k_1 \) increases say to 0.4, every one-in-twenty trials (95% confidence interval) the treatment time will be over 4 times longer than that predicted from the mean rate constant. If the coefficient of variation in \( k_1 \) is 0.77, then every one-in-
three trials (standard error) the treatment time will be about 4-fold the expected value. The expected value of \( t/t_{1/2} \) for 90% of the parent compound conversion is 3.32 (or \( \log[t/t_{1/2}] = 0.52 \)).

![Figure 21. Impact of Error in the Rate Constant on the Probability of Extended or Reduced Treatment Times Relative to the Prediction (Based on 90% Conversion of Parent Compound)](image)

Errors resulting in behavior lower than the expected value on Figure 22 result in faster treatments than that expected. Greater predictive reliability here results in more accurate bids, potentially lower costs, and greater bioremediation process competitiveness.

This analysis has assumed that the error in the rate constant is a normal distribution. In reality, the distribution describing the rate constant is a one-sided bounded distribution. This is intuitive if one thinks about the biotransformation rate constant (a negative number) as it approaches zero biotransformation rate. In this type of distribution, the standard deviation between the mean value and zero is less than the standard deviation.
between the mean value and $-\infty$. For this reason, the confidence envelope shown as positive time deviations on Figure 22 actually represent limiting worst cases. The 1:20 Performance Envelope case at a coefficient of variation of 0.4 would be something less than 4-fold longer than the expected value and would not have as severe an economic impact as that proposed here. However, it might still likely be an economic catastrophe for the environmental biotechnology service company or his client.

2.5 DESORPTION/DIFFUSION AS A BIOTRANSFORMATION RATE LIMITATION

The biodegradative kinetic testing reported above can be thought of as a measure of the optimal intrinsic biotransformation kinetic potential (IBKP) in MGP soils. This is because the PAH compound is added to the test already dissolved in a solvent and has maximum availability to the aqueous phase and the microorganisms. Partitioning of the compound to microorganisms, to soil, to non-aqueous phase liquid (NAPL), to organic solid or plastic phases, and other solids present clearly occurs and competes with the biotransformation mechanism. As indicated in Figure 2 for toluene, biotransformations are expected to increase PAH solubility and reduce solid partitioning. This type of test minimizes the loss to sorption processes and maximizes removal by biotransformation.

Strong evidence that PAH partitioning onto the non-aqueous phases and "slow" diffusion/desorption rates into the aqueous phase system can be rate-controlling and therefore may be very important in the field-scale MGP bioremediation process was developed in a separate experiment. An MGP soil was preloaded with $^{14}$C-naphthalene and was allowed to stand for a day. The soil was then added to a continuously-stirred reactor with a filter insert to contain the solids as described in § 3.0 and was run with a continuous feed containing non-radiolabeled ($^{12}$C) naphthalene. Results from this batch test are presented in Figure 22.
The radiolabeled naphthalene preloaded on the MGP soil biotransformed with a half-life of about 11 hours, while the unlabeled naphthalene steadily fed to the reactor in the aqueous feed biotransformed with a half-life of under 6 seconds.

Several transport mechanisms can be proposed that offer explanations for the nature and physics of a slow desorption/diffusion process. However, it is very difficult (usually beyond our experimental capabilities) to conclusively identify the responsible mechanism in a real MGP soil. In systems analysis lingo, this is known as a system model which is not unique. The deterministic model describing the slow process is difficult to uniquely identify. For these reasons, the protocols proposed in this work have the purpose of quantifying the slow process phenomenologically—in terms of time response instead of elucidating mechanisms deterministically.

2.6 CONCLUSIONS

- A small vial-type batch laboratory test protocol can be used to perform treatability studies on PAH degradation in MGP soils. These kinetic results are
idealized intrinsic biotransformation kinetic potentials (IBKP) that may be compared in a relative way, but should not be used directly to scale-up processes.

• The batch IBKP tests make use of radiolabeled parent PAH compounds and their conversion products in a solvent-soil extract. Results appear to have improved accuracy and precision relative to similar tests based on chemical analysis of PAHs. This is likely a result of the minimization of "slow" sorption/desorption processes caused by rapid and bioavailable addition of the test substrate and the ability to discriminate the added substrate from the indigenous substrate.

• The resulting improved batch IBKP precision leads to good correlations between the specific PAH batch biotransformation rates, and the soil total PAH composition.

• When coupled with a protocol (still needing development and verification) to measure the slow desorption/diffusion rates of PAHs from MGP soils, the IBKP results could be used to select the most suitable MGP soils for bioremediation. These treatability studies have as a purpose, selection of the best and rejection of the worst MGP soils for bioremediation. They will not alone be suitable for scale-up.

• New scale-up protocols are needed to permit the use of standardized treatability test rate results to predict actual PAH bioremediation performance in a given field-scale test. These can only be developed when the laboratory method results are coupled to actual field-scale remediation results. This concept is presented in this report but development and verification of the scale-up protocols must await future work.

• PAH biotransformation rate processes in batch PAH bioremediations are non-elementary and/or time-variant. Prediction of batch field-scale time-
concentration profiles will require a new level of sophistication in resolving this complex behavior. This might take the form of a new artificial intelligence system that returns the "best" empirical Rate Equation from experimental time-concentration profile data.

- The need for improved accuracy in the rate constants, leading to better field-scale predictive reliability of design and performance mandates greater accuracy and precision in the initial and final PAH concentrations and sampling time measurements. Also, attention is required to the test design so that a minimum allowable PAH bio-conversion is met.

- The use of added radiolabeled PAH as a test compound minimizes the effects of diffusion/desorption on the measured removal and tends to experimentally isolate biotic conversion processes from the mass transfer kinetic effects. In addition, this approach appears to diminish the coefficient of variation of the analytical results and offer the potential of greater statistical significance with a lower number of replicate samples or tests.

- Second-order rate constants are formulated by dividing the first-order constants by a microbiological parameter. The overall precision in second-order rate constants may be easily improved by increasing the number of replicate microbiological analyses to some number greater than three.
3.1 DYNAMIC MICROBIAL SYSTEMS ANALYSIS

Earlier sections of this report have dealt with the complexity of batch PAH biotransformation processes in MGP soils. These have been found to be non-elementary and/or time-variant and therefore are difficult to reliably scale-up with classical deterministic modelling approaches. This problem is not unique to PAH bioremediation and alternative approaches have been identified in the field of Systems Engineering.

Two broad divisions of the analysis of complex systems are the "direct problem" and the "inverse problem" approaches to systems analysis (Figure 23). The direct problem approach describes the traditional approach taken by engineers and scientists when a process is to be modelled and its performance or behavior predicted. Any system can be conceptualized as being composed of three components: the process, input to the process, and output from the process. The direct problem deals with formulation of a model from prior knowledge to describe behavior of the process, creation of a "known" input into that process, and subsequent calculation of the "unknown" output from that process. Simply put, two of three "variables" are "known" and the third--the "unknown" output--is calculated.

This game may be played in other ways, too. Where the objective is to formulate the model or the input as the unknown, the "inverse problem" approach is employed. In Figure 23, three such inverse problems are noted: one seeking the best design, one seeking the nature of an input to give a given output, and one seeking the model of the process. In the third case, the inverse problem approach is called system identification, and with known inputs and outputs from a "black-box" system, the process model itself is extracted.
System identification methods are numerous and can be divided into two categories: nonparametric and parametric model methods. In non-parametric methodology, little or no prior knowledge on the system structure is needed and it truly is handled as a black box. The type of mathematical model generated is a phenomenological model that maps the known input into the known output. Clearly the resolution of this type of model depends on the ability to create or monitor the input and output signals without interference from other noise from process disturbances.
The mathematical model developed in this approach has as its independent variable, time, and as its dependent variable(s), the input and output variables. An example of such a model might be as follows:

$$\frac{d^2 \theta}{dt^2} + \gamma \frac{d\theta}{dt} + \omega^2 \theta = 0$$

This is the deterministic equation of motion for a damped harmonic oscillator, such as a real pendulum. In this case, the parameters have physical meaning ($\gamma$ is the damping coefficient and $\omega$ is related to the pendulum's weight and length, and $\theta$ is the angle of the pendulum at an instant of time.

In another case, however, observation and mathematical analysis of an unknown system's response to a disturbance (we'll assume that we're observing a biological damped harmonic oscillator system, but we don't know this in advance) may give an equation of similar form:

$$a \frac{d^2 y}{dt^2} + b \frac{dy}{dt} + cy = u(t)$$

Here $u$ is the measured input variable and $y$ is the measured output variable. The system identification method tells us that the descriptive model is a linear sum of $y$ and its first two time derivatives (each proportional to some parameter). If the system is simple and well-understood, perhaps the coefficients can be interpreted with physical meaning. If the system is not well understood, the physical meaning of the coefficients may remain very obscure.

One non-parametric system identification method used widely in engineering is the frequency-response method of Bode.18, 19 An input signal is created that is sinusoidal in form and is generated at a known frequency. This is done a number of times at various frequencies while the output is measured. For linear systems, the complex system phenomenological model can be
graphically taken from a plot of the output response as a function of input frequency. For non-linear systems (where the phenomenological model has at least one term where variables of time or its derivatives are a product or quotient with another such time-based variable), the model extracted represents a linearized approximation of one of the states of the "true system model" and for time-variant systems, the model represents a linearized snapshot of the system response at the time of the experiment.

The parametric approach also has numerous possible methodological alternatives. One potent method when a significant level of knowledge about the system exists deals with writing the best possible deterministic model about the system's behavior, then knowing the system input and output, solving for one or more parameters that aggregate variation and uncertainty. For example, Equations A.22-A.40 in Appendix A present an unsteady-state material balance model of PAH fates in a continuous-stirred tank reactor. All parameters can be measured or estimated except for the rate of PAH biotransformation. By knowing the system input (feed concentration) and output (reactor effluent concentration), the biotransformation rate can be estimated. This approach is called parameter estimation and in this case is based on a deterministic material balance on the PAH in the reactor.

Dynamic microbial systems analysis is a very powerful approach to be taken in the analysis of the complexities of PAH biotransformation in MGP soils. In general, it offers the potential for:

- A standardized approach for testing microbial systems to identify critical or dominant mechanisms for further targeted research,

- A predemonstration testing and evaluation protocol to classify microbial processes for reliability and effectiveness under environmental disturbances,
• A protocol leading to the identification of regimes of stability and robustness to disturbances that offer the potential for optimization by managing the nature of the disturbance,

• A protocol to assess system adaptability and its importance to structure and activity, and

• A dynamic experimental protocol that provides an experimental platform for testing and evaluation of new monitoring approaches and process improvement schemes.

This section describes the application of two system identification methods for the study of dynamic responses of biodegradation in soil systems. The work is focused on developing techniques for monitoring and analyzing continuous flow, PAH degrading soil systems. Parameter estimation techniques provide the framework to extract biotransformation rate constants from time-series sampled data. This rate information represents idealized intrinsic biotransformation kinetic potential estimates for continuous systems. In addition, Bode non-parametric frequency-response techniques are used to establish the stability and robustness of PAH-degrading systems. This approach has the potential of identification of phenomenological dynamic response “Rate Equations” for use in design and scale-up, however this was unnecessary in this work since satisfactory first-order rate behavior was experimentally determined for most experiments.

3.2 DESCRIPTION OF EQUIPMENT

This section has a focus on the development of a continuous liquid flow, complete mix reactor for studying PAH degradation in soil slurries. The system and operating protocols were developed to maintain a homogeneous soil slurry at constant, controlled operating conditions (temperature, oxygen flow, agitation, feed flow, pH, etc.). On-line monitoring of offgas substrate concen-
trations under continuous operating conditions was developed previously. The entire system evolved and was optimized over the course of this three-year project.

The experimental apparatus was conceptually adapted by DiGrazia in his Ph.D. research for analysis of PAH degradation in soil slurries from the design used by Blackburn. The apparatus was composed of four main components (shown schematically in Figure 24): the feed supply system, the bioreactor system, the offgas analysis system, and the computer control system.

The feed system consisted of refrigerated containment vessels for storage of feed materials and two, computer controlled positive displacement pumps. All feed lines and other surfaces exposed to feed materials were stainless steel, glass, or poly-TFE in order to minimize adsorption of PAH to reactor materials.

The bioreactor system used for dynamic analysis of naphthalene degradation in soil slurries consisted of a direct drive, continuous flow bioreactor with a 0.75 L working volume. The reactor was equipped with temperature control, air flow control, and dissolved oxygen measurement modules. The reactor system was modified for experimentation with soil slurries (Figure 25) by using an inner reaction vessel fabricated with sintered stainless steel. The inner vessel was designed to contain reactor solids while allowing effluent to filter through the reactor walls.
Offgas from the reactor was routed to a computer controlled sampling valve. Gas samples were analyzed using gas chromatography with packed column and flame ionization detector. All components of the offgas sampling system were heated to 140°C to minimize adsorption of PAH to surfaces of the offgas sampling system.
The pump flow rates, sample valve, gas chromatograph, and data acquisition were controlled using a personal computer. The computer program was capable of controlling up to eight complete reactor systems simultaneously with the option of producing constant, square wave, sinusoidal, or sine sweep patterns of flow control.

Several experiments were performed to establish operating protocols for microbial systems analysis of the soil slurry reactor system. See Experiments T2.04, T2.05, T2.07, T2.09, T2.10, T2.11, T2.12, T2.14c, T2.15, T2.16, T2.17, T2.24, T2.25, T2.29, T2.30, T2.31, and T2.33 in Appendix B.

3.3 RESULTS OF PRELIMINARY STUDIES

Early continuous system trials were performed in order to begin system development and define operating parameters for the latter experiments with
MGP soil. In general, these early tests were done with PAH-spiked uncontaminated soil and the later tests (§ 3.4) were done with MGP Soil A. Because of different and evolving system designs and operating procedures, comparison of results beyond those reported here should only proceed after the specifics of each experiment are reviewed (see Appendix B).

3.3.1 MATERIALS

Two model soils were used in the reactor studies. An uncontaminated Etowah silt loam surface soil from a site in Knoxville, Tennessee was used to produce soil extract feed material for all of the experiments. This soil was also used in preliminary reactor and vial experiments. Table 10 describes some physical and chemical characteristics of the Tennessee soil. The second model soil was a sample provided by GRI from a manufactured gas plant (MGP) site. The soil has been characterized by ReTeC (Soil A) and the details of the analysis have been presented previously.2

The feed material for the continuous experiments was prepared by hot water extraction from the Tennessee soil. After extraction, solids were removed by continuous centrifugation followed by pressure filtration. Large quantities were prepared and stored at 4°C to eliminate batch-wise variation over the course of the project. The total organic carbon in the feed before addition of naphthalene averaged 50 mg/L. Naphthalene was added to the feed by saturating the solution at 50°C and allowing the naphthalene to crystallize out of solution while cooling to 4°C.

The inoculum used for the mixed culture soil slurry experiments was prepared from a series of batch enrichments from MGP soil slurries supplemented with naphthalene, phenanthrene, and anthracene, and combined with an archived set of samples from PAH-exposed freshwater microcosms.21 The final enrichment cultures were spiked with additional PAH-degrading strains, frozen and stored at -80°C to provide inocula for these and future studies.
Table 10. Characteristics of Uncontaminated Tennessee Control Soil

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:1 soil:water)</td>
<td>5.9</td>
</tr>
<tr>
<td>Total Carbon, %</td>
<td>0.8</td>
</tr>
<tr>
<td>Cation Exchange Capacity, meq/100 g (pH 7)</td>
<td>6.5</td>
</tr>
<tr>
<td>Mechanical Analysis, %</td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td>24</td>
</tr>
<tr>
<td>Coarse Silt</td>
<td>18</td>
</tr>
<tr>
<td>Silt</td>
<td>33</td>
</tr>
<tr>
<td>Clay</td>
<td>25</td>
</tr>
<tr>
<td>Fe, % as free Fe oxide</td>
<td>1.9</td>
</tr>
<tr>
<td>Clay-sized Minerals*</td>
<td>HKMQG</td>
</tr>
</tbody>
</table>

*K = kaolinite, H = hydroxyinterlayer vermiculite, M = mica, Q = quartz, G = gibbsite, in relative order of abundance.

3.3.2 OPERATING PARAMETERS

Various amounts of soil were added to the reactor to determine the operating range for maintaining a homogeneous soil slurry in the reactor. The agitation rate and air flow rate were varied to determine the effect of these parameters on the physical soil distribution in the reactor. The air flow rate had little effect on the soil distribution. Agitator rates above 400 r.p.m. were sufficient to maintain a homogeneous soil slurry, as visually observed.

The oxygen mass transfer rates from the air feed to the reactor liquid were determined over a range of air flow and agitation rates (Experiment T2.07). Experiment T2.12 established the oxygen requirements for microbial cultures used in the soil slurry studies. The results indicated that for all agitation rates above 200 r.p.m. and air flow rates above 75 mL min⁻¹, the reactor could supply sufficient oxygen to maintain an aerobic system.
To ensure adequate oxygen supply and soil distribution, and to minimize stripping of PAH from the system, the operating air flow rate was chosen as 100 mL min\(^{-1}\) with an agitation rate of 500 r.p.m. This represents a specific power input of 12 hp/1000 gal (2400 W/m\(^3\)).

### 3.3.3 PARTITION COEFFICIENTS

Parameter estimation of biotransformation rates in a defined system requires complete characterization of parent compound fates, such that the only unknown component in the mass balance is the term representing removal due to degradative processes. Characterization of the abiotic fate of the parent compound is accomplished by operating the reactor system in the same manner as when conducting biotic experiments, while suitably suppressing biological activity. In the continuous stirred tank reactor (CSTR) soil slurry system, biological activity was inhibited by using a 0.05% mercuric chloride in soil extract solution to "poison" the active population. In addition, the air feed stream was replaced with a pure nitrogen stream in order to eliminate aerobic biotransformations. This procedure, coupling two independent methods to inhibit biological activity, maximized the chances that biotransformation was eliminated in the soil slurry reactor.

As presented in Equation A.22, the major fates of naphthalene in the soil slurry reactor are stripping in the offgas, sorption to solids, and removal in the effluent. Two types of frequency response experiments were used to characterize the abiotic fates of naphthalene in the reactor system. First, sinusoidal perturbations were induced in the feed naphthalene concentration fed to an abiotic reactor with no soil. This data was used to characterize the stripping process. Second, the same protocol was used to study stripping in the soil slurry system. Previous data (Experiment T2.14c) suggested that soil in the reactor system did not affect the stripping process. Comparison of the data from the stripping and soil slurry experiments were compared to test this hypothesis.
Stripping of naphthalene from agitated biological treatment systems can be modeled as an equilibrium process by Equation A.33,\textsuperscript{8, 22, 23} where the gas concentration is proportional to the liquid concentration. Sorption of naphthalene to soil can also be modeled as an equilibrium process by Equation A.34. These models can be substituted into Equation A.31 which can be solved analytically to give Equation A.36.

Equation A.40 results when biotic rate processes are set to zero and relates to the abiotic (sterile or biologically inactive) case. Laplace transformation of Equation A.40 results in a transfer function for the abiotic system (Equation A.45). The amplitude ratio and phase angle of the output waveform with respect to the input waveform for a transfer function of this form can be expressed as Equations A.46 and A.47, respectively.\textsuperscript{24}

The values of the unknown stripping and sorption parameters were therefore determined from abiotic dynamic experiments. Each individual data set (one for each frequency tested) was fit to Equation A.40 using nonlinear regression techniques.

\[
\left(1 + \frac{K_p}{\rho_s \nu} \right) \frac{dC_A}{dt} = \frac{F}{V} C_A^0 - C_A \left(\frac{F}{V} + K_g\right) \quad \text{Eqn. A.40.}
\]

All parameters are known save $K_p$ and $K_g$. These results with the Henry’s Law Constant calculated from $K_g$ are reported in Table 11.

If the amplitude ratio and phase angle of the response are plotted against the input frequency of the feed perturbations (Figure 26), it is observed that the data clearly fit two experimental curves. The upper curve in Panel A of Figure 26 represents the amplitude ratios for the abiotic experimental runs with no soil. The upper curve in Panel B represents the phase angle for the abiotic-no soil runs. Fitting the amplitude ratio data to Equation A.46 (with A.43 and A.44) and fitting the phase angle data to Equation A.47...
(also with A.43 and A.44) results in a sorption parameter of zero and stripping parameters of 0.0079 and 0.0110 for the 10°C and 20°C experiments, respectively.

\[ A_R = \frac{\beta}{\sqrt{\alpha^2 + \omega^2}} \quad Eqn. A.46. \]

\[ \phi = \tan^{-1}\left( -\frac{\omega}{\alpha} \right) \quad Eqn. A.47. \]

\[ \beta = \frac{F}{V\left(1 + \frac{\nu A p}{\rho_s}\right)} \quad Eqn. A.44. \]

\[ \frac{C_A}{C_{A0}} = C = \frac{\beta}{s + \alpha} \quad Eqn. A.45. \]

The lower curves represent the experimental runs with both Tennessee and MGP soils. Similar mathematical analysis as described above, but using a non-linear, two parameter curve fitting technique, results in a stripping parameter value of 0.0079 and a sorption parameter value of 12.1 for the 10°C experiments, and a stripping parameter value of 0.0110 and a sorption parameter value of 11.7 for the 20°C experiments. This analytical approach is an alternative to direct use of a material balance (Equation A.40) and permits extraction of abiotic parameters from a series of perturbation/response experiments. These alternate parameter values correspond favorably with the results presented in Table 11.

The literature values also compare favorably with the experimental values for the Tennessee soil. However, the similarly low sorption parameter values for the MGP soil were not expected since the fraction of organic
carbon in the MGP soil is much higher than that of the Tennessee soil. Equation A.34 predicts that the sorption parameter should increase with an increase in the soils organic carbon content. This increase in organic carbon may be related to the non-aqueous phase tar component. In this case, it is likely that an increase in the sorption parameter would only be observed over long time frames since sorption into this phase may be rate-limited by the relatively long time required for PAHs to diffuse through the tar itself. The impact of the increased organic carbon of the MGP soil would therefore not be observed in these experiments which focus on the faster rate processes.

Table 11. Determination of Stripping and Sorption Abiotic Rate Constants in Perturbed CSTR Experiments (Temperatures: 10 and 20°C)

<table>
<thead>
<tr>
<th>Type of Soil</th>
<th>Perturbation Cycle Period (hr)</th>
<th>Henry's Law Constant at Temperature (unrtless x10^-3)*</th>
<th>Sorption Constant, Ks at Temperature (mL/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10°C</td>
<td>20°C</td>
</tr>
<tr>
<td>No Soil Added</td>
<td>4</td>
<td>8.1</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>7.7</td>
<td>10</td>
</tr>
<tr>
<td>Uncontaminated</td>
<td>4</td>
<td>7.9</td>
<td>11</td>
</tr>
<tr>
<td>Tennessee Soil</td>
<td>8</td>
<td>8.1</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.0</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>9.8</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>6.5</td>
<td>15.1</td>
</tr>
<tr>
<td>MGP Soil</td>
<td>4</td>
<td>7.2</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.4</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>9.1</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>7.7</td>
<td>9.9</td>
</tr>
</tbody>
</table>

* Unitless Henry's Law Constant is, for example (mg/L in the gas) / (mg/L in the liquid). It is not the same as a unitless mole fraction ratio.
3.3.4 KINETIC STABILITY AND ROBUSTNESS

The initial CSTR protocol called for operation of the reactor system at 20°C, an air flow rate of 100 mL min⁻¹, and a soil extract feed with 14 mg L⁻¹ maximum naphthalene concentration. The initial CSTR runs at these conditions established that the naphthalene degradation rates were consistently high enough to reduce the reactor liquid concentration below the detection limit of 0.5 μg L⁻¹ (Experiments T2.15, T2.16). Frequency response systems identification techniques could not be used to characterize dynamic system behavior since the output signal was below detection. Also, parameter estimation or resolution of Rate Equations could not proceed without measured
concentration data. Since the naphthalene analysis used was already very sensitive (\(-0.5 \mu g L^{-1}\)) and further improvements in chemical analysis sensitivity would be at the expense of rapid time-series sampling (\(-10\) samples/hr), bioactivity reduction was investigated as a strategy for increasing the reactor liquid concentration permitting microbial systems analysis.

The first attempt to reduce the rates of naphthalene biotransformation was to reduce the reactor temperature. The temperature in two soil slurry reactors operating for several weeks at 20°C was reduced to 10°C to reduce biotransformation activity (Experiment T2.18). The reactors were operated for a period of one week before the feed naphthalene concentration was varied sinusoidally (cycle period 1 hour). The naphthalene level remained above the limit of detection throughout this period, but subsequently fell below the detection limit after the cycles in naphthalene feed concentration were stopped. This is an experimental indication of time-variance in the biotransformation process and may also be related to a change of microbial system state (state bifurcation).

The reactor temperature was further reduced to 4°C. The reactor liquid naphthalene concentration increased to a level near 100 \(\mu g L^{-1}\) and remained there for a period of one week, when the experiment was ended. Although this represented a significant increase in the reactor naphthalene concentration into a range where the analytical scheme would be effective, with a feed concentration of \(-10 mg L^{-1}\) and effluent concentrations of \(-100 \mu g L^{-1}\), over 99% of the naphthalene in the liquid feed stream was biotransformed (Experiment T2.18). That these high biotransformation rates continued even at low temperatures was surprising but later confirmed.

In order to verify that the loss of naphthalene even at low temperatures was, in fact, due to aerobic biotransformation and not some other obscure biotic or abiotic fate mechanism, the experimental system was modified such
that the air feed stream could be combined with a pure nitrogen feed stream. The gas flow rate to the reactor was kept constant while the fraction of oxygen in the feed was gradually reduced.

The biotransformation rate was affected only when oxygen was almost totally eliminated from the feed gas, thus forcing the dissolved oxygen concentration in the reactor below 1 mg L\(^{-1}\) (Experiment T2.20). When a pure nitrogen gas feed was used, biodegradative activity ceased, as indicated by a rise in the reactor naphthalene concentration to a steady-state level equal to the calculated level corresponding to abiotic stripping as the only fate mechanism for naphthalene removal. While the approach of limiting the terminal electron acceptor in the experiments was experimentally viable and reductions of activity could be so controlled, it was unclear how to compare the results with the batch IBKP results and to field applications.

A different approach was then considered to reduce biotransformation activity and increase reactor liquid concentration. A hypothesis was possible that one cause for the high naphthalene biotransformation rates was that naphthalene was essentially the sole carbon source. By supplying alternative carbon sources to the system, naphthalene would not be a requirement for cell survival and naphthalene biotransformation rates might be reduced by supplying additional carbon sources to the system.

The first experiment involved using MGP soil in place of Tennessee soil. The MGP soil has multiple PAH contaminants which would serve as alternative carbon sources to the system. This change of soil type had no measurable effect on system performance (Experiment T2.22) as indicated by naphthalene concentrations remaining below analytical detection limits. The second experiment involved adding phenanthrene and anthracene to the feed stream to provide additional organic carbon. This also had no effect on system behavior (Experiment T2.26). Finally, yeast extract, peptone, glucose
(YEPC) media was used in place of buffered soil extract as the feed material. Again, this media, rich in organic carbon, had no measurable effect on naphthalene biotransformation rates (Experiment T2.28).

While this series of experiments was unable to identify operating conditions where the liquid concentration signals were detectable, indirect results showed that naphthalene biotransformation was surprisingly persistent and robust in the face of changes in concentrations of naphthalene and other carbon compounds, temperature and oxygen levels.

3.4 NAPHTHALENE BIOTRANSFORMATION IN THE CSTR TEST SYSTEM

Several choices were possible in the design and operation of the test reactor. Given that a continuous mode is desirable, the feed can contain both liquid and soil, or liquid alone. In the second case, one must decide whether to use: a flow-through strategy where the soil is permitted to leave the reactor with the liquid effluent or an alternative strategy where the soil is contained or separated from the liquid effluent and recycled back to the reactor.

Continuous feeding of dry and easily-flowing solids to a reactor has precedent. However, MGP soils are neither dry nor well-behaved. It was believed that semibatch manual additions in a small reactor could not be reproducibly accomplished over the extended operation planned. Further, the addition might cause an important dynamic disturbance interfering with reactor behavior. Deciding that this approach would itself require a research project beyond the time available for this work, continuous MGP soil addition was abandoned.

Since a focus of the study was to maintain a long-term system operation to study dynamic response, the alternative of permitting the soil to wash out of the reactor was impractical. The half-life concentration of the soil in the reactor
would be the same as the residence time—no more than a day or so. Also, a decline in the soil concentration might strongly impact the state of the system in ways difficult to interpret.

Capturing or containing the soil was the best operational and simulative choice. A simplistic approach, similar to that used in lab activated sludge studies, was first selected. This made use of an operational strategy of daily manual separation of the soil from the collected effluent by centrifugation, then returning the solids back to the system. Named "Manual Soil Recycle" in this report, many of test results were collected in this mode.

Because of evidence that the manual soil recycle option itself introduced large disturbances into the analysis and because the operation was labor-intensive, an improved alternative using a filter reactor insert was developed, tested and used in the final frequency-response experimental campaign.

3.4.1 RESULTS FROM MANUAL SOIL RECYCLE MODE

The first naphthalene frequency response experiment is outlined in Experiment T2.15 in Appendix B. The reactor was operated for a period of one week under steady feed conditions in order to acclimate the degrading culture to the system. After the first week, the feed naphthalene concentration was varied sinusoidally (cycle period 24 hours) for an additional week. The reactor liquid response to the naphthalene feed perturbations is shown in Figure 27.

The reactor liquid naphthalene concentration varied periodically between 0 and 14 µg L⁻¹, three orders-of-magnitude below the feed concentration which ranged from 0 to 14 mg L⁻¹. This experiment demonstrated that frequency-response methodology could be applied to study biotransformation in soil slurry reactors, however, problems were identified. The increased naphthalene concentration in the reactor liquid coincided with the daily maintenance of the reactor, as well as with the increase in feed con-
centration. During reactor maintenance the solids were collected from the effluent and returned to the reactor. It was difficult to determine whether the apparent sinusoidal response of the reactor was due to system response to the feed perturbations or to daily maintenance disturbances.

After the 24-hour cycle, the reactor feed naphthalene concentration was kept constant, the reactor naphthalene concentration again fell below the detection limit.

All subsequent experiments at 20°C had similar results—the reactor liquid naphthalene concentration remained below the detection limit of 0.5 μg L⁻¹. A series of steps were taken to inhibit biodegradation of naphthalene in the soil slurry system, as outlined in § 3.3.4. After a reduction in reactor temperature to 10°C, the reactor liquid naphthalene concentration increased above the detection limit, indicating that the naphthalene biotransformation rate in the system had also been reduced.

After a one-week acclimation period, a one-hour feed cycle was initiated. This one-hour cycle is shown as the “baseline” in both panels of Figure 28. Two reactors were evaluated. The first reactor (Panel A) was operated in such a manner that the feed liquid naphthalene concentration was varied sinusoidally, which also resulted in a variation in total organic carbon (TOC) to the system. The second reactor (Panel B) had a constant naphthalene concentration in the feed while only the non-naphthalene organic carbon was varied. The response of the two reactors to the feed perturbations is shown in Figure 28.

Three key observations can be made: 1) the reactor naphthalene concentration in both reactors varied periodically with the same frequency as the feed perturbation, 2) a significant increase in reactor naphthalene concentration was observed following reactor maintenance each 24 hours, and 3) the amplitude of the reactor liquid naphthalene concentration in Panel
A is greater than that of Panel B. This indicates that the periodic response of the reactor in the previous experiment (24-hour cycle, 20°C) was due to the combined effects of daily reactor maintenance and the feed perturbations.

![Graph](image)

**Figure 27. CSTR Response to Sinusoidal Perturbation in Feed Naphthalene Concentration (24-Hour Cycle Period, Uncontaminated Tennessee Soil, Manual Recycle, Experiment T2.15)**

After reducing the temperature in the reactors to 4°C, the reactor liquid naphthalene concentration rose to a level of 100 µg L\(^{-1}\) and remained there for a period of one week. Slurry samples were taken from Reactor 1 at time points at 20°C, 10°C, and 4°C and tested in offline batch assays (similar to the batch IBKP proposed in § 2.0) to verify the biotransformation activity observed in the CSTR reactors (Experiment T2.21).
Figure 28. CSTR Response to Sinusoidal Perturbation in Feed Naphthalene Concentration, Panel A, and Non-Naphthalene Organic Carbon, Panel B (1-Hour Cycle Period, Uncontaminated Tennessee Soil, Manual Recycle, Experiment T2.18)

Biotransformation rate constants in both the CSTR reactor and the batch assays were calculated based on the assumption that the biotransformation was first-order with respect to reactor liquid naphthalene concentration. Mineralization rates were based upon $^{14}$CO$_2$ accumulation whereas biotransformation rates were calculated from $^{14}$C-naphthalene disappearance data. The results are presented in Table 12. It is clear that the naphthalene transformation rates in the batch assays were
orders-of-magnitude lower than those observed in the CSTR reactor. Also note that biotransformation and mineralization activity observed at 4°C was verified in the batch assay, although the rate was much slower.

Table 12. Pseudo-First- and Second-Order Rate Constants from CSTR Reactor Studies and Batch Naphthalene Biotransformation and Mineralization Assays

<table>
<thead>
<tr>
<th>CSTR Interval</th>
<th>CSTR Temp (°C)</th>
<th>Gene Probe Positive Bacteria</th>
<th>Batch Assay Overall Pseudo-First-Order Rate Constant (day⁻¹)</th>
<th>Batch Assay Overall Pseudo-Second-Order Rate Constant (x 10⁻⁷ g cfu⁻¹ day⁻¹)</th>
<th>CSTR Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>k₁ᵇ</td>
<td>k₁ᵐ</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>nd</td>
<td>3.8</td>
<td>nd</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>-3.8</td>
<td>2.3</td>
<td>-7.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>-1.8</td>
<td>1.1</td>
<td>-9.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>-0.7</td>
<td>0.2</td>
<td>-1.5</td>
</tr>
</tbody>
</table>

* designates an in vivo-based rate constant  
* no gene probe positive bacteria  
nd not determined

In a separate experiment, a 30 g sample of soil was loaded with ¹⁴C-naphthalene and added to the reactor (Experiment T2.23). The soil slurry was sampled periodically for ¹⁴C-naphthalene and offgas sampling was used to monitor ¹⁴CO₂ production. The results are presented in Figure 22. The results indicate that the biotransformation (and mineralization) of the ¹⁴C-naphthalene occurred at a rate three orders-of-magnitude lower than
the naphthalene in the liquid feed stream. This is strong evidence supporting the hypothesis that desorption/diffusion of the naphthalene from the soil is a slow and rate-limiting step in the overall biodegradation process.

![Graph showing bacterial concentrations](image)

Figure 29. Total Heterotrophic Bacteria and nah Gene Probe-Positive Bacteria Concentrations in CSTR Tennessee Soil Experiments (Panel A, Naphthalene Perturbation; Panel B, Non-Naphthalene Organic Carbon Perturbation)

In a number of sequential experiments on the same soil sample (Experiments T2.16, T2.18, T2.19, T2.20, T2.21, T2.23) biological samples were taken for analysis of total viable and naphthalene degrading cells. Both
one-hour perturbation periods and unperturbed intervals were included in these experiments. The results are presented in Figure 29. No significant differences in microbial population levels were seen between temperature ranges, as indicated by analysis of variance (95% confidence interval). The changes of microbial concentrations within a temperature treatment may be significant and result from environmental disturbances.

3.4.2 REACTOR FILTER INSERT

In light of the fact that the washout and subsequent recycling of the soil from the reactor may have affected the performance of the system and manual soil recycle was highly labor intensive, a new reactor was designed to contain the soil in the reactor while allowing liquid effluent to flow through the reactor. This was accomplished by fabricating a reactor filter insert from sintered stainless steel. A soil slurry was contained in the reactor while liquid effluent flowed through the pores (0.4 to 4 μm) of the reactor. The liquid was then collected in an outer sleeve in order to control the reactor slurry volume (Figure 25). Experiments were designed and implemented to verify that this design was appropriate for the study of naphthalene biotransformation in the soil slurry. Once the apparatus was tested, the frequency response protocol was implemented in this newly designed system.

There is an obvious difference between the two sets of continuous experiments discussed—in the early “manual-recycle” experiments, the soil was allowed to flow out of the system and was subsequently recycled; in the later experiments with the reactor insert the soil did not leave the reactor. This may have resulted in differences in biomass activity which may complicate direct comparison of the data from the two types of reactor experiments.

The manual recycle system may result in a different microbial community within the reactor, which may effect cellular activity. The centrifugation process might not have been efficient enough to guarantee the recycle of all
bacteria with preferential loss of the unflocculated bacteria. The mean cell residence time and possibly the population distributions of the two systems were likely different. It is unknown at this time what effect these events had on the operating state of the system, however, it is possible that the microbial system states of the two reactor systems were different. Biotic PAH conversion data should be compared between these two systems only with caution.

### 3.4.2.1 EARLY SYSTEM OPERABILITY STUDIES

A sample of the sintered metal was obtained from the manufacturer in the form of a cylinder for testing for our application. A mass transfer coefficient was experimentally determined (Experiment T2.24 and Equations A.48-56) which verified that the hydrostatic pressure in a reactor fabricated from the same metal was sufficient for operation at the desired flow rates. Visual inspection of the filtrate indicated that the sintered metal would retain the soil while allowing liquid to pass through the wall.

A reactor filter insert was then fabricated and tested with an active biomass/soil slurry to test for blockage of the pores with biomass. No blockage occurred, however, microscopic observation of the effluent established that some biomass, not soil particles, was present. It was concluded that the reactor could be operated for an extended period using the established protocol without blocking with soil or biomass. However the capture efficiency of microorganisms in the reactor may have been less than complete.

### 3.4.2.2 FREQUENCY-RESPONSE ANALYSIS

A series of frequency response experiments were performed over a range of feed perturbation cycle periods from 1 to 24 hours (Experiment T2.34). Each experiment was initiated by inoculating the reactor with MGP soil and the standard inoculum, and allowing the system to acclimate under continuous flow conditions, constant naphthalene feed concentra-
tion, for a period of four days. Following the acclimation period, a sinusoidal variation between 0 and 14 mg/L in naphthalene feed concentration was initiated.

The reactor offgas samples were processed periodically throughout the cycle to determine reactor naphthalene concentrations. During three of the experiments, $^{14}$C-naphthalene was injected into the reactor to verify the degradative activity observed by sampling for $^{14}$CO$_2$ in the offgas, and for $^{14}$C-metabolites and $^{14}$C-naphthalene in the reactor. Reactor slurry samples were taken daily for biomass analysis of both total viable cells and naphthalene-degrading genotype.

Figure 30. CSTR Response to Sinusoidal Perturbation in Feed Naphthalene Concentration (Panel A, 1-Hour Cycle Period; Panel B, 12-Hour Cycle Period; MGP Soil, Filter Insert, Experiment T2.34)
An example of the typical reactor response, for a short (1 hour, Panel A) and long (12 hours, Panel B) cycle, is shown in Figure 30. The reactor concentrations were typically three orders of magnitude lower than the feed concentrations, indicating that the conversion of naphthalene was consistently greater than 99%. The rate of biodegradation at each sample point was calculated using Equation A.39 and plotted versus reactor liquid concentration in Figure 31.

![Figure 31. Naphthalene Biotransformation Rate vs. Naphthalene Reactor Liquid Concentrations (Panel A, 1-Hour Cycle Period; Panel B, 12-Hour Cycle Period; MGP Soil, Filter Insert, Experiment T2.34)]

While there is considerable scatter in the data, a linear relationship exists between naphthalene reactor liquid concentration and the naphthalene biotransformation rate. Data for the 1-, 4-, and 24-hour cycles are similar to Figure 31, Panel A, all can be fit with an elementary first-order
kinetic model (with a zero y-intercept). Data for the 2-, 8-, and 12-hour cycles are similar to Figure 31, Panel B, which can also be fit with a first-order model. However, there is an apparent minimum liquid naphthalene concentration below which the rate of naphthalene biotransformation is zero (i.e., a model of form: \( R = k(C-C_{\text{min}}) \)). The rate constants and minimum concentrations are presented in Tables 13 and 14.

Table 13. Summary of Interval Continuous IBKP Pseudo-First-Order Naphthalene Biotransformation Rate Constants and Reactor Liquid Concentrations

<table>
<thead>
<tr>
<th>Cycle Period (hr)</th>
<th>Interval Pseudo-First-Order Biotransformation Rate Constant, ( k_1 ) b* (day(^{-1}))</th>
<th>Naphthalene Reactor Liquid Concentrations (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>-324</td>
<td>-348</td>
</tr>
<tr>
<td>2a</td>
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</tr>
<tr>
<td>24</td>
<td>-418</td>
<td>-475</td>
</tr>
</tbody>
</table>

Table 13. Summary of Interval Continuous IBKP Pseudo-First-Order Naphthalene Biotransformation Rate Constants and Reactor Liquid Concentrations
Table 14. Summary of Interval Continuous IBKP Pseudo-Second-Order Naphthalene Biotransformation Rate Constants

<table>
<thead>
<tr>
<th>Cycle Period (hr)</th>
<th>Interval Pseudo-Second-Order Biotransformation Rate Constant ((10^{-7} \text{ g cfu}^{-1} \text{ day}^{-1}))</th>
<th>Based on Heterotrophic Bacteria, (k_{2bh}^*)</th>
<th>Based on nah Gene Probe Positive Bacteria, (k_{2bp}^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Mean</td>
<td>Max.</td>
</tr>
<tr>
<td>1</td>
<td>-2.35</td>
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<td>-4.51</td>
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<td>2a</td>
<td>-1.58</td>
<td>-2.18</td>
<td>-3.17</td>
</tr>
<tr>
<td>2b</td>
<td>-2.32</td>
<td>-3.24</td>
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</tr>
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<td>4</td>
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<td>-10.5</td>
<td>-16.7</td>
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<tr>
<td>8c</td>
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<td>-12.5</td>
<td>-19.8</td>
</tr>
<tr>
<td>12</td>
<td>-11.1</td>
<td>-14.5</td>
<td>-20.4</td>
</tr>
<tr>
<td>24</td>
<td>-5.85</td>
<td>-8.33</td>
<td>-12.5</td>
</tr>
</tbody>
</table>

Interestingly, the first-order rate results (Table 13) show very high kinetic activity at the 8-hour perturbation period. This tends to increase the CV for all of the perturbation experiments (CV = 1.0). Both second-order rate constants (Table 14) tend to even out these 8-hour peaks with the resulting improvement in statistical dispersion (CV for \(k_{2bh}^* = 0.69\) and CV for \(k_{2bp}^* = 0.86\)). The second-order constant based on nah gene probe enumerations may have a higher dispersion as related to that based on heterotrophic bacteria because of experimental difficulty in counting colonies on gene probe plates from these continuous experiments. Colonies were irregularly shaped and mucoid leading to difficulty in colony discrimination. In this case, colonies from heterotrophic plate counts were much more distinct.
The appearance of an apparent threshold concentration in the first-order rate constant formulation (Table 13) below which no degradation seems to occur for one of the 8-hour and the 12-hour cycles was unexpected. Such a limit in substrate concentration has been proposed by certain investigators. However, since this concentration limit was not observed in numerous other experiments, experimental artifacts leading to this result must also be considered. The fact that these concentration limits occurred in successive experiments in only one reactor support the possibility that these are experimental artifacts.

Fourier spectral analysis was used to determine the amplitude and phase angle of the sinusoidal reactor response with respect to the input signal. The results are presented along with comparative results from an activated sludge petrochemical wastewater treatment system in Figure 32.

The CSTR MGP soil slurry system's amplitude ratio is relatively constant with the exception of the response at the 8-hour cycle. Here three experiments were run because the first 8-hour experiment produced anomalous results and because interesting behavior was seen in prior work in activated sludge near this frequency. All three experiments produced interesting but different responses than those at other frequencies.

A duplicate run was performed at the 2-hour period to see if non-reproducible results as seen in the 8-hour test replicate frequency response tests were typical. Results of the 2-hour duplicate runs were similar to one another. A possible conjecture is that near the 8-hour period the system is inherently less robust and microbial system state bifurcations resulting from some difference in the start-up procedure leads to a different population distribution with greatly different (and in this case improved) naphthalene biotransformation capability.
The phase angle frequency response behavior may be a clearer indicator of the critical frequencies for system response. In the activated sludge work, the phase angle began to decrease without apparent bound as the frequency increased. Two observations may be made: a critical system time constant appears at around 12-hour periods and the system is a "non-minimal-phase" dynamical system. Systems that have dead times in the naphthalene carbon processing metabolic pathways would exhibit this non-minimum phase behavior.

The soil slurry phase angle response is very different than the activated sludge response. No "turning frequency" is evident down to the experimental limit of 1-hour perturbation period. Therefore, in the soil slurry system, the critical frequency appears to be much less than the activated sludge system and therefore, the response stability is greater than the activated sludge system. No statement is possible regarding the presence or absence of naphthalene metabolic dead times in the soil slurry system. In terms of the response to naphthalene concentration perturbations, the soil system appears to be more stable than the activated sludge system. This is true even though the soil system appears to undergo time-variant state changes, at least at certain perturbation frequencies.
The concept of system time-variance and state changes is further enforced when the actual concentration response data for one of the 8-hour replicates is seen (Figure 33). In this figure, the reactor liquid con-
centration in the relaxed condition early in the experiment where the naphthalene feed concentration is constant also is relatively constant. When the 8-hour period perturbation begins, the reactor liquid concentration first responds, then drops precipitously to the detection limit. This behavior was seen in two of the three 8-hour replicates and in a number of early manual soil recycle mode experiments.

![Graph showing reactor liquid concentration and feed concentration over time]

Figure 33. System Time-Variance and Possible State Bifurcation Under 8-Hour Period Perturbations (MGP Soil, Filter Insert). Reactor Liquid Concentration--Open Squares, Feed Concentration--Solid Line

The phase angle shift can also be observed by plotting the rate of biotransformation versus the naphthalene feed rate. The two rates are nearly equal for all of the data, however, when the output signal is out of phase with the feed cycle, an apparent hysteresis is observed (Figure 34, Panel A). This type of plot magnifies even small phase angle changes.
Figure 34. Naphthalene Biotransformation Rate vs. Naphthalene Feed Rate (Panel A, 1-Hour Cycle Period; Panel B, 12-Hour Cycle Period; MGP Soil, Filter Insert, Experiment T2.34)

Figure 35 shows the results of the naphthalene radiolabel fate analysis. In contrast to the batch reactor experiment described in § 2.5, the radiolabel naphthalene was added as a pulse into the otherwise continuous liquid feed rather than pre-sorbed on soil. The results of this continuous experiment indicate that the naphthalene is oxidized immediately following addition to the reactor, as indicated by the insignificant amount of naphthalene present at the first sampling point. The radiolabel shows up in the aqueous phase as polar metabolites (30%) and $^{14}$CO$_2$ (20%). The remaining fraction (50%) is assumed to be resident in the solids contained in the reactor insert.
These results verify that the rates of biotransformation are very rapid when the naphthalene is available in the liquid phase. It is also apparent that complete degradation of the metabolic intermediates was much slower than the initial biotransformation. The hydraulic residence time of this reactor was 18 hours, sufficient to mineralize all but 30% of the naphthalene's polar metabolites. Longer residence times may lower the distribution of polar metabolites due to lessening of the reactor washout, but it is clear that these compounds are more resistant to further biotransformation as compared to biotransformation of naphthalene itself. Further characterization of these polar compounds and their toxicity remains a priority for future work.

Figure 35. Radiolabeled Analysis of Naphthalene Fates in Perturbed CSTR Experiment (1-Hour Cycle Period, MGP Soil, Filter Insert, Experiment T2.34)
3.5 CONCLUSIONS

- Kinetic evaluation of naphthalene biotransformation in the continuous Intrinsic Biotransformation Kinetic Potential test (IBKP) established that the rate of biotransformation in this reactor system with the MGP soil studied can be modeled with a first-order rate equation. Use of frequency-response system identification approaches to generate "black box" phenomenological response models were, in this case, unnecessary.

- Naphthalene biotransformation in the continuous IBKP for the soils tested was a robust process, resistant to perturbations in substrate concentrations, other organic concentrations, temperature, and dissolved oxygen concentrations.

- The continuous IBKP, combined with frequency-response analysis, offers a practical method for establishing the stability and robustness of biodegradation in a specific process. Even though time-variant, state-change bifurcations seemed to be possible, naphthalene biotransformation in the soil slurry system studied here was more stable to naphthalene concentration changes than an activated sludge system degrading naphthalene in a complex petrochemical wastewater.

- The continuous IBKP test as developed in this work preferentially characterizes "fast" sorption/desorption mass transfer processes in specific wastes. This is seen in the similar comparative abiotic responses between MGP soil and uncontaminated PAH-spiked soil. The method is insensitive to "slow" mass transfer processes since addition of MGP soil in a frequency-response mode is experimentally challenging. "Slow" sorption/desorption kinetics may be more easily characterized in batch abiotic experiments suggested in § 3.0.
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4.0 OBSERVATIONS ON NAPHTHALENE BIOREMEDIATION KINETICS

4.1 COMPARISON OF KINETIC RATE DATA BETWEEN SIMILAR REACTOR CONFIGURATIONS

When overall pseudo-first-order kinetic rate constants based on only the first and final concentrations are compared between similar reactor configurations (in this case between batch IBKP tests) very significant correlations relating the rate constants may be possible. As discussed in § 2.3, a correlation between five MGP soils using the total PAHs analytically determined led to a good relationship predicting the pseudo-first-order biotransformation rate constant for phenanthrene. It is also significant that correlations based on total carbon and/or microbial counts (pseudo-second-order) for these tests were poor. Here, the best relationships led to first-order predictions based on total PAH measurements.

4.2 PREDICTION OF BATCH TIME-CONCENTRATION PROFILES

Batch biotransformation of PAHs in MGP soils are generally not first-order or even elementary in terms of rate expressions. Further, they may be time-variant. *Prediction of time-concentration profiles other than the endpoint will be prone to significant error and is not recommended.* Empirical rate expressions must be developed and verified if reliable time-concentration predictions are desired.

4.3 COMPARISON OF BATCH AND CONTINUOUS RESULTS

Table 15 presents a summary of the naphthalene biotransformation kinetic results from this study, and for comparison, from a prior study in an activated sludge system operating on a non-MGP petrochemical wastewater.⁹
In general, all kinetic rate constants (interval) for continuous tests exceed those of the batch tests (overall). On a pseudo-first-order basis, the differences are very significant with the batch test 1-3 orders of magnitude slower than the continuous test. The pseudo-second-order kinetics based on total heterotrophic bacterial counts compare more favorably with the rate constants for the batch and the continuous IBKP (1-4 hour perturbation period) being the same. The continuous IBKP second-order kinetic constants based on gene probe assays are presented but should not be considered too critically since the gene probe counts in the continuous system were experimentally difficult to enumerate—colony morphology was mucoid, irregular and difficult to discriminate.

Table 15. Summary of Naphthalene Biotransformation Rate Constants

<table>
<thead>
<tr>
<th>Test System</th>
<th>Ref.</th>
<th>( k_1 ) or ( k_1^b ) (day(^{-1}))</th>
<th>( k_2^{bh} ) or ( k_2^{bh*} ) (x10(^{-7}) mL cfu(^{-1}) day(^{-1}))</th>
<th>( k_2^{bp} ) or ( k_2^{bp*} ) (x10(^{-7}) mL cfu(^{-1}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch IBKP</td>
<td>This work</td>
<td>-1.66</td>
<td>-3.0</td>
<td>-13</td>
</tr>
<tr>
<td>MGP Soil A</td>
<td>This work</td>
<td>-592</td>
<td>-7.1</td>
<td>-76</td>
</tr>
<tr>
<td>Continuous IBKP</td>
<td>This work</td>
<td>-261</td>
<td>-2.8</td>
<td>-38</td>
</tr>
<tr>
<td>MGP Soil A(^a)</td>
<td>This work</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous IBKP</td>
<td>MGP Soil A(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated Sludge(^d)</td>
<td></td>
<td>0</td>
<td>-144</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Average of all perturbed intervals (does not include data from relaxed intervals).
\(^b\) Average of fast perturbation intervals—1-4 hour periods.

For comparison between different system configurations and applications, the batch IBKP overall rate constant results may give a reasonable second-order estimate of the average minimum continuous IBKP interval rate constant results (those experiments perturbed with periods less than 4 hours). First-order comparisons between different system configurations and applica-
tions are disparate and are not recommended for design and scale-up. The potential exists for using second-order lab-scale batch kinetic results to estimate the "worst-case" performance of lab-scale continuous systems. Coupled with new approaches to relate the lab-scale results with field-scale results, reliable "worst-case" field performance estimates may be possible.

Because of enhanced robustness and stability, it is possible that continuous processes may be more reliably scaled-up than batch processes. The tendency toward time-variant state changes under perturbed conditions may negate this possibility.

4.4 SECOND-ORDER KINETICS

Differences between experiments in the number of microbial PAH degraders is likely to have an effect on the overall degradation kinetics. The overall pseudo-first-order biotransformation rate constant from the batch experiments was reported as -1.66 day\(^{-1}\) which is significantly slower than the interval results from the CSTR study, -592 day\(^{-1}\) or the activated sludge study, -144 day\(^{-1}\).

Apparently, the continuous process leads to both higher total and PAH-degradative bacterial concentrations in the CSTR reactor than does the batch IBKP system. This supports the hypothesis that active bacterial cell concentration is a critical parameter needed to predict PAH degradation rate constants in MGP soil bioremediation systems.

The pseudo-second-order rate constants presented in Table 15 are likely to be better activity parameters for system comparison and scale-up than the pseudo-first-order rate constants presented in the same table. However, their use in scale-up requires an a priori capability for predicting either the total heterotrophic or genotypic bacterial concentrations in given field-scale systems. This is beyond current the state-of-the-art, but should be given a high R&D priority in microbial ecology for further work.
4.5 MASS TRANSFER

Mass transfer of PAH from the soil to the aqueous phase where it would be most available to the microorganisms can have a large, possibly rate-controlling effect on the rates of degradation. A clear indication of this is evident when reviewing the work done by ReTeC. Both methods used for studying PAH degradation were batch methods which resulted in comparable treatment endpoints. However, the two test systems, a completely-mixed batch slurry reactor and a batch soil pan reactor, gave vastly different results in terms of rates of degradation. The rates in the completely-mixed reactor were much faster than in the soil pans, indicating that the PAH was more readily available in the completely-mixed system. Experiments in both batch and continuous systems in this work corroborate that mass transfer limitations can be rate-limiting. However, negligible PAH biotransformation rates were also established suggesting that biotic activity could also be rate-limiting.

It is difficult to directly compare ReTeC's data with data from this study, largely because the ReTeC work reported conventionally-analyzed PAHs aggregated by ring number, and did not report comparable microbial concentrations. It is interesting to speculate that because ReTeC studied batch systems, the differences in their kinetics may have arisen through desorption/diffusion limitations while the differences in the kinetics (batch vs. continuous IBKP) in this work may have resulted from both desorption/diffusion limitations as well as enhanced microbial concentrations arising from the continuous flow-through system.

4.6 NAPHTHALENE BIOTRANSFORMATION IN SOILS VS. WASTEWATER

Naphthalene biotransformation (interval pseudo-first-order rate constants, Table 15) in perturbed, continuous MGP soil slurry systems is faster than those in perturbed, continuous activated sludge systems (-592 vs. -144 day\(^{-1}\), respectively). However, the activated sludge system's response to feed perturbations of less than or equal to 12-hour periods was significantly less than that of the soil system.
The MGP soil systems here were able to respond to perturbations without significant changes in amplitude ratio or phase angle down to 1-hour perturbation periods. While being cautious not to disregard differences in the feed composition (the activated sludge feed was a concentrated petrochemical wastewater), and not withstanding the apparent time-variant state bifurcations evident in the soil slurry testing, one may speculate that there exists a significantly greater naphthalene biotransformation stability in the soil system than that found in the activated sludge system. Frequency response methodology appears to be useful in classifying and comparing very different naphthalene-degrading systems.

4.7 FIELD-SCALE PROCESSES

The very fast rate behavior of all of these lab-based experiments as compared to field-scale rates reported in the literature lead to the conclusion that currently lab-scale testing may be useful for performance comparison across systems and applications, but that direct scale-up of rate information may be dangerous. The impact of scale on kinetics is well-established in chemistry and chemical engineering and in these disciplines, separate “scale-up” relationships have been developed over the years to aid in the scale-up process. Analogous relationships for scale-up of bioremediation processes for environmental applications do not exist. Their development should be a priority.

High pseudo-first-order rates of continuous processes relative to batch processes and the apparent enhanced stability and robustness of continuous soil slurry bioremediation processes suggests that continuous processes (“open processes” in terms of the reactor liquid) should be considered for field-scale development. This effect may be related to the dilution and wash-out of soluble inhibiting metabolites and/or organisms in a continuous system vs. their accumulation in a batch system. Enhanced predictability and performance of continuous processes over batch processes may make up for the added capital and operating costs of treatment of a continuous process.

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Treatability and Scale-up Protocols for Polynuclear Aromatic Hydrocarbon Bioremediation of MGP Soils

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5.0 REFERENCES


APPENDIX A. SUMMARY OF CALCULATIONS AND MODELS

A-1. BATCH AND CONTINUOUS REACTOR DESIGN MODELS

Derivations for the equations in this section are presented in Levenspiel. The zero- and first-order Rate Equations are, respectively:

\[-r_A = -\frac{dC_A}{dt} = k_z\]  \hspace{1cm} Eqn. A.1.

\[-r_A = -\frac{dC_A}{dt} = k_1C_A\]  \hspace{1cm} Eqn. A.2.

In any constant volume batch reactor, the overall design equation is:

\[t = \frac{C_{A_0}}{k_z} \int \frac{dX_A}{-r_A}\]  \hspace{1cm} Eqn. A.3.

where the fractional conversion of compound A is \(X_A\):

\[X_A = \frac{C_{A_0} - C_A}{C_{A_0}}\]  \hspace{1cm} Eqn. A.4.

The integrated Design Equations for zero- and first-order Rate Equations, therefore respectively become:

Batch, zero-order form:

\[C_{A_0} - C_A = C_{A_0}X_A = k_z t\]  \hspace{1cm} Eqn. A.5.
Batch, first-order forms--

\[- \ln \frac{C_A}{C_{A_0}} = k_1 t \quad \text{Eqn. A.6.}\]

\[\frac{C_A}{C_{A_0}} = e^{-k_1 t} \quad \text{Eqn. A.7.}\]

\[- \ln (1 - X_A) = k_1 t \quad \text{Eqn. A.8.}\]

\[X_A = 1 - e^{-k_1 t} \quad \text{Eqn. A.9.}\]

In a perfectly-mixed, constant volume, continuously stirred tank reactor (CSTR), the design equation is found by using the form for a backmix reactor:

\[\tau = \frac{C_{A_0} X_A}{r_A} \quad \text{Eqn. A.10.}\]

CSTR Zero-order form--

\[C_{A_0} - C_A = C_{A_0} X_A = k_2 t \quad \text{Eqn. A.11.}\]

CSTR First-order form--

\[\frac{C_{A_0}}{C_A} = 1 - X_A = \frac{1}{1 + \tau k_1} \quad \text{Eqn. A.12.}\]
A-2. PROPAGATION OF ERROR TO THE KINETIC RATE CONSTANTS

The parameters that vary as a function of experimental errors in the above Design Equations are C\textsubscript{AO} (the initial concentration of compound A for a batch reactor or the feed concentration of A for a continuous reactor), C\textsubscript{A} (the concentration of A at time, t, in a batch reactor or the effluent or concentration in a continuous reactor), t (the treatment time in a batch reactor), and \tau (the hydraulic residence time in a continuous reactor).

Assuming that the chosen Rate Equations apply and that each rate constant is indeed a constant, the estimation of errors in these parameters on the rate constants, k\textsubscript{2} and k\textsubscript{1}, can be estimated by assuming values for positive and negative error in the parameters and calculating the resulting rate constants. The magnitude of the relative error in the rate constant can then be calculated as follows:

\[
\text{Magnitude of Rate Constant Relative Percent Error} = \left| \frac{k - k_{\pm}}{k} \right| \quad \text{Eqn. A.13.}
\]

Assuming the simplest possible case where all removal from this batch experiment is biotic in nature (no important abiotic processes are at play), where the reactor is homogeneous (no error is incurred due to sampling from different locations at one time), and where the biotransformation rate equation is either zero- or first-order; the estimation of experimental errors on the resulting rate constant is straightforward.

Eight types of error propagation can occur when considering negative and positive error in three variables. These cases are shown in Table A-1.
For the abiotic experiments, \( X=0 \) and \( R=0 \), resulting in further simplification to the form:

\[
\left( 1 + \frac{K_P}{\rho_s} \right) \frac{dC_A}{dt} = \frac{F}{V} C_{A0} - C_A \left( \frac{F}{V} + K_g \right) - \frac{R}{V}
\]   \(\text{Eqn. A.39.}\)

With a sinusoidal feed input of naphthalene of the form:

\[
C_{A0} = \frac{C_0}{2} (1 + \cos(\omega t))
\]   \(\text{Eqn. A.41.}\)

Equation A.40 can then be solved analytically to give:

\[
C_A = e^{-\alpha t} C_A |_{t_0} + \frac{C_0 \beta}{2\alpha} (e^{-\alpha (t-t_0)} - 1) + \frac{C_0 \beta}{2(\alpha^2 + \omega^2)} [\omega \sin(\omega t) - \alpha \cos(\omega t) + e^{-\alpha (t-t_0)} (\alpha \cos(\omega t) - \omega \sin(\omega t))]
\]   \(\text{Eqn. A.42.}\)

where

\[
\alpha = \frac{F + QH}{V \left( 1 + \frac{V X}{\rho_s} \right)}
\]   \(\text{Eqn. A.43.}\)

and

Laplace transformation of Equation A.40 yields:
The amplitude ratio and phase angle for a transfer function of this form are:

\[ \beta = \frac{F}{V(1 + \frac{\nu \cdot \omega}{s})} \quad \text{Eqn. A.44.} \]

\[ \frac{C_s}{C_{s0}} = G = \frac{\beta}{s + \alpha} \quad \text{Eqn. A.45.} \]

\[ \begin{align*}
AR &= \frac{\beta}{\sqrt{\alpha^2 + \omega^2}} \quad \text{Eqn. A.46.} \\
\Phi &= \tan^{-1}\left(-\frac{\omega}{\alpha}\right) \quad \text{Eqn. A.47.}
\end{align*} \]

A-5. FLOW EQUATIONS FOR THE SINTERED METAL REACTOR INSERT

The liquid mass flow through the reactor insert is proportional to the surface area and the pressure gradient:

\[ \text{Mass Flux} = KA_2 \Delta p \quad \text{Eqn. A.48.} \]

\[ = K \int_0^d 2\pi r \Delta p dz \quad \text{Eqn. A.49.} \]

\[ = 2\pi r K[\int_0^l p_o dz + \int_l^d (p_o - p_i) dz] \quad \text{Eqn. A.50.} \]

\[ p_o - \rho_w z \quad \text{Eqn. A.51.} \]

\[ p_i = \rho_w(z - l) \quad \text{Eqn. A.52.} \]
Term(G) =
\[-\left(\frac{\text{Effluent Flow Rate}}{\text{Cell Concentration}}\right) \left(\frac{\text{Compound Effluent Cell Concentration}}{\text{Concentration}}\right) = -FXC_A^b\]
Eqn. A.29.

Term(H) = \left(\frac{\text{Reactor Offgas Flow Rate}}{\text{Concentration}}\right) \left(\frac{\text{Compound Reactor Offgas Concentration}}{\text{Concentration}}\right) = -QC\]
Eqn. A.30.

Substitution of Equations A.23 through A.30 into Equation A.22 yields:

\[\frac{d}{dt}(1'\cdot C_A) - \frac{k'}{\rho_s} \frac{d}{dt} (\frac{C_A^b}{H}) + 1' \cdot \lambda \cdot \frac{d}{dt} (\frac{C_A^b}{H}) = FC_A - \frac{1'}{\rho_s} \cdot F \cdot \frac{k'}{\rho_s} \cdot C_A^b - FXC_A^b - QC_A - R\]

The reactor liquid volume is directly related to soil concentration and slurry volume:

\[1' = 1' \left(1 - \frac{k'}{\rho_s}\right)\]
Eqn. A.32.

The compound reactor offgas concentration is proportional to the compound reactor liquid concentration:

\[C_A^b = HC_A\]
Eqn. A.33.

The compound reactor soil concentration is proportional to the compound reactor liquid concentration:
The compound reactor biomass concentration is proportional to the compound reactor liquid concentration:

$$C_A^b = \frac{f_L \rho_b K_{ow}}{\rho_l} C_A = K_b C_A \quad Eqn. A.35.$$ 

Substitution of Equations A.32 through A.35 into Equation A.31 yields:

$$\frac{dC_A}{dt} = \frac{F}{V} C_A^0 - \frac{F}{V} C_A (1 + K_s W + K_b X + K_q) - \frac{R}{V} \quad Eqn. A.36.$$ 

where

$$K_s = \frac{K_p - 1}{\rho_i} \quad Eqn. A.37.$$ 

and

$$K_q = \frac{QH}{F} \quad Eqn. A.38.$$ 

Equation A.36 is valid for all experiments performed in the CSTR. For experiments with the sintered metal reactor insert, terms F and G of Equation A.22 are zero. Also, the liquid fraction in the effluent is one, and the soil concentration is constant. This results in simplification of Equation A.36 to the form:
In these calculations, \( C_{(\text{hexane extract})\, t=0} \) was taken as the radiolabel concentration in the "time=0" hexane extract samples and not the expected concentration based on what was added.

**A-4. MODEL FOR DYNAMIC SYSTEMS ANALYSIS OF A CSTR SOIL SLURRY REACTOR**

\[
\begin{align*}
\text{Term (A)} = & \\
& \left( \frac{\text{Time Rate of Change}}{\text{Volume}} \right) \left( \text{Compound Mass Accumulation in the Reactor Liquid} \right) = \frac{dV}{dt} C_A \\
E_{qn. A. 23}
\end{align*}
\]
Term (B) =
\[
\left( \frac{\text{Time}}{	ext{Rate of Change}} \right) \left[ \frac{\text{Slurry Volume}}{\text{Soil Volume Factor}} \left( \frac{\text{Compound Reactor Soil Volume Factor}}{\text{Concentration}} \right) \right] = \frac{d}{dt} \left[ V \left( \frac{W}{\rho_s} \right) C_s^t \right] = V \frac{d}{dt} \left( \frac{W C_s^t}{\rho_s} \right)
\]

Term (C) =
\[
\left( \frac{\text{Time}}{	ext{Rate of Change}} \right) \left[ \frac{\text{Slurry Volume}}{\text{Reactor Cell Volume Concentration}} \left( \frac{\text{Compound Reactor Cell Volume Concentration}}{\text{Concentration}} \right) \right] = \frac{d}{dt} \left( V x C_s^t \right) = V x \frac{d}{dt} \left( C_s^t \right)
\]
Eqn. A.25.

Term (D) =
\[
\left( \frac{\text{Feed Rate}}{\text{Compound Feed Concentration}} \right) = F C_{A0}
\]

Term (E) =
\[
- \left( \frac{\text{Effluent Flow Rate}}{\text{Effluent Liquid Volume Fraction}} \right) \left( \frac{\text{Effluent Concentration}}{\text{Compoud Liquid Effluent Concentration}} \right) = - F \frac{V}{V} \frac{C_s}{C_s}
\]
Eqn. A.27.

Term (F) =
\[
- \left( \frac{\text{Effluent Flow Rate}}{\text{Soil Volume Fraction}} \right) \left( \frac{\text{Effluent Soil Concentration}}{\text{Compound Soil Effluent Concentration}} \right) = - F \frac{V}{\rho_s} \frac{C_s}{C_s}
\]
Eqn. A.28.
Table A-1. Types of Error in Three-Parameter Design Equations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
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</thead>
<tbody>
<tr>
<td>( C_{A0} )</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>( C_A )</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>( t ) or ( \tau )</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

A-3. CALCULATION OF PSEUDO-FIRST-ORDER BIOTRANSFORMATION AND MINERALIZATION RATE CONSTANTS FROM LABORATORY-SCALE EXPERIMENTS

For biotransformation rates, the average value of the three hexane extract scintillation counts was recorded as a time series. A pseudo-first order biotransformation rate constant can be calculated for each time increment by application of Eqn. A.6 to each time interval. This "interval-based" rate constant carries a superscript * and is defined as follows:

\[
 k_i^* = -\frac{\ln \left( \frac{C_{A_i}^{t_{n+1}}}{C_{A_i}^{t_{n+1}}} \right)}{t_n - t_{n-1}} \quad Eqn. A.14
\]

If the above equation is applied to intervals of time series data, the initial concentration is the concentration at the beginning of the sampling interval and the rate constant is calculated accordingly. This approach lends itself to a recursive formula where the conversion is calculated for each interval after the preceding concentration is established.

\[
 C_A |_{i=n} = C_A |_{i=n-1} e^{-k_i^* t_n} \quad Eqn. A.15
\]

An alternative approach is to use a formula to calculate the rate constant where the initial concentration is the starting concentration for the experiment.
but the conversion is the conversion between each successive interval. In practice, these rate constants apply to rate equations where concentrations are calculated from the starting concentration and do not require a recursive approach.

\[ X_A = X_A|_{t=0} + X_A|_{t=1} + \ldots + X_A|_{t=n} \quad \text{Eqn. A.16.} \]

\[ X_A|_{t=n-1} = X_A|_{t=0} - X_A|_{t=n} = \frac{C_A|_{t=n} - C_A|_{t=n-1}}{C_A|_{t=n}} \quad \text{Eqn. A.17.} \]

\[ X_A|_{t=n-1} = \frac{C_A|_{t=n-1} - C_A|_{t=n}}{C_A|_{t=n}} \quad \text{Eqn. A.18.} \]

\[ k_1^p = -\frac{\ln(1 - X_A|_{t=n})}{t_n - t_{n-1}} \quad \text{Eqn. A.19.} \]

The pseudo-first-order mineralization rate constants were calculated by application of Eqn. A.19 on the concentration of labeled carbon atoms.

\[ k_1^m = -\frac{\ln(1 - X_{\text{CO}_2}|_{t=n})}{t_n - t_{n-1}} \quad \text{Eqn. A.20.} \]

where

\[ X_{\text{CO}_2}|_{t=n-1} = \frac{C_{\text{CO}_2}|_{t=n} - C_{\text{CO}_2}|_{t=n-1}}{C_{\text{CO}_2(\text{hexane extract})}|_{t=0}} \quad \text{Eqn. A.21.} \]
Substitution of Equations A.51 and A.52 into A.50 yields:

\[ Mass\ Flux = 2\pi r_p w K \left[ \int_0^l zdz + \int_l^d zdz \right] \quad Eqn. A.53. \]

Solving:

\[ Mass\ Flux = 2\pi r_p w K \left( ld - \frac{l^2}{2} \right) \quad Eqn. A.54. \]

\[ \frac{dV}{dt} = 2\pi r K \left( ld - \frac{l^2}{2} \right) \quad Eqn. A.55. \]

Maximum flow rate occurs when \( I = d \):

\[ \left. \frac{dV}{dt} \right|_{max} = \pi r K d^2 \quad Eqn. A.56. \]

A-6. NOMENCLATURE

AR is the ratio of the output signal amplitude and the input signal amplitude.

\( A_s \) is the surface area of the filter insert.

B is the concentration of biomass present.

\( C_A, C_A^s, C_A^b, \) or \( C_A^v \) are the concentrations of compound A in a given phase or compartment. Superscripts \( s, b, \) and \( v \) represent the concentration of compound A in the soil, biomass and offgas phases or compartments, respectively, while no subscript indicates the concentration in the aqueous liquid phase.

\( C_{A0} \) is the starting concentration in the case of a batch reactor system or the feed concentration in the case of a continuous reactor system.

\( C_0 \) is the maximum feed concentration.

d is the diameter of the filter insert.

F is the volumetric flowrate of the feed stream to a continuous reactor.
$f_L$ is the weight fraction of lipids in biomass.

$f_{OC}$ is the weight fraction of organic carbon in soil.

$H$ is the Henry's Law constant in units of weight concentration in the gas over weight concentration in the liquid.

$K$ is a mass flow constant.

$K_b$ is the overall partition coefficient of compound A into biomass.

$K_g$ is the overall partition coefficient of compound A into the reactor offgas.

$K_{OW}$ is the octanol-water partition coefficient of compound A.

$K_p$ is the overall partition coefficient of compound A in soil.

$k_1$, $k_1^b$, $k_1^m$; $k_2$, $k_2^b$, or $k_2^m$ represent the first-order or second-order overall rate constants with subscripts signifying first-order, or second-order Rate Equations and superscripts $b$ or $m$ signifying biotransformation or mineralization, respectively. These may be evaluated over many time intervals or a single interval depending on the nature of the calculation. An asterisk "*" superscript signifies that the constant is being evaluated over a single time interval and is based upon the compound concentration at the beginning of that interval for a batch reactor configuration.

$k_z$ is the rate constant for a zero-order rate equation.

$k_1\_\_\_$ is the rate constant evaluated when its parameters are assigned certain errors that propagate through to the constant.

$I$ is the height of the filter insert.

$Q$ is the volumetric flowrate of gas leaving a continuous reactor.

$R$ is the mass removal rate of compound A arising from biotic mechanisms.

$r_A$ is an expression or rate equation for the change in compound A over time.

$s$ is the independent Laplace variable.

$t$ is either elapsed time or specified times in a time series.

$V$ is the volume of the slurry (liquid and solids) in a soil slurry reactor.

$V_L$ is the liquid volume in a soil slurry reactor.

$W$ is the weight of the soil present.

$X_A$ is the conversion of compound A. This may be evaluated over various time intervals as noted by $X_A|_{\tau}$, for example.

$\Delta P$ is the pressure drop across the filter insert.
$\Phi$ is the phase angle between the input signal and the output signal.

$\rho_s$ is the density of the soils present.

$\rho_b, \rho_L$ are the densities of the biomass and the reactor liquid, respectively.

$\tau$ is the reactor residence time in a continuous reactor.

$\omega$ is the frequency of oscillation.
APPENDIX B. DESCRIPTIONS OF EXPERIMENTS

[This section includes descriptions of the nature and design of each major experiment cited in this report. These are intended to enable the comparison of the experimental methods from case-to-case. This Appendix is extensive and is available on request under separate cover from the GRI Project Manager.]