

The Numerical Modeling of Microbial Reductive Dechlorination: Monod Kinetic Parameter Estimation and Uncertainty Analysis

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Abstract

This dissertation examines pitfalls commonly encountered when fitting models describing the sequential microbial reductive dechlorination (MRD) of chlorinated aliphatic hydrocarbon (CAH) compounds, and proposes enhancements to the conventional parameter estimation methodology that result in order-of-magnitude precision and accuracy improvements.

One of the most common oversights in the literature is fitting an MRD model that assumes no experimental mass loss, which a review showed is usually around 5 to 50 percent due to sampling and sorption to rubber septa. A series of numerical model-fitting experiments indicated that fitting a closed-system model to batch reactor experiments with mass loss imparts 5 to 120 percent error to estimated Monod kinetic parameters. Mass loss is well described by a simple first-order model formulation for short term batch reactor or microcosm experiments, but mass loss in longer term experiments is better described by a model that simulates sorption into the septum as a planar diffusion process.

Another potential pitfall is employing a model regression scheme that does not leverage information about measurement uncertainty. Laboratory measurements from microcosm or batch reactor experiments can vary greatly in precision and magnitude, but the standard least-squares model fitting approach does not take these factors into consideration. A superior weighted-least-squares estimation technique was employed to fit an MRD model to a set of batch reactor experiments with varying concentrations of per- and polyfluoroalkyl substances (PFAS), and the likelihood maximization inherent in this technique enabled statistical inference regarding the inhibitive effects of PFAS on MRD.

A series of Monte Carlo experiments (comprised of more than one million independent MRD model fits to synthetic datasets) indicated that the conventional experimental design (triplicate of batch reactors) often leads to observations unsuitable for even order-of-magnitude parameter estimation. An improved experimental design with the same number of batch reactors and sample analyses but varied initial CAH concentrations was confirmed to result in median confidence interval width decreases of 70 percent, averaged across all fitting parameters. Classical regression analysis techniques commonly used to calculate marginal confidence intervals contained true parameter values much less frequently than their nominal confidence levels. A Metropolis algorithm was shown to return more reliable parameter interval estimates.

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1 Introduction

1.1 In-Situ Bioremediation as a Remedy for Widespread Chlorinated Solvent Contamination

Widespread groundwater and soil contamination caused by misuse of the chlorinated solvents tetrachloroethene (also known as perchloroethylene, or PCE) and trichloroethene (TCE) has resulted in a pernicious threat to human health and an ongoing remediation challenge. PCE is primarily utilized as a drycleaner solvent, although its production peaked in the mid-1990s as laundering technology improved.^{1,2} TCE's primary industrial use has historically been as a cleaning and degreasing agent; its domestic production declined precipitously in the 1970s with the advent of the Clean Air Act and toxicological studies showing that it caused cancer in mice.^{1,3} Uncontrolled land disposal of spent chlorinated solvents was not regulated until the early 1980s; after passage of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), PCE and TCE became two of the most commonly detected Superfund site contaminants.¹ As of 2017, PCE and TCE were present at 57 and 51 percent of the 1,854 hazardous waste sites proposed for inclusion on the EPA National Priorities List.^{4,5} If subsurface contamination is left untreated, these compounds can enter the human body via vapor intrusion into indoor air (see Figure 1.1).

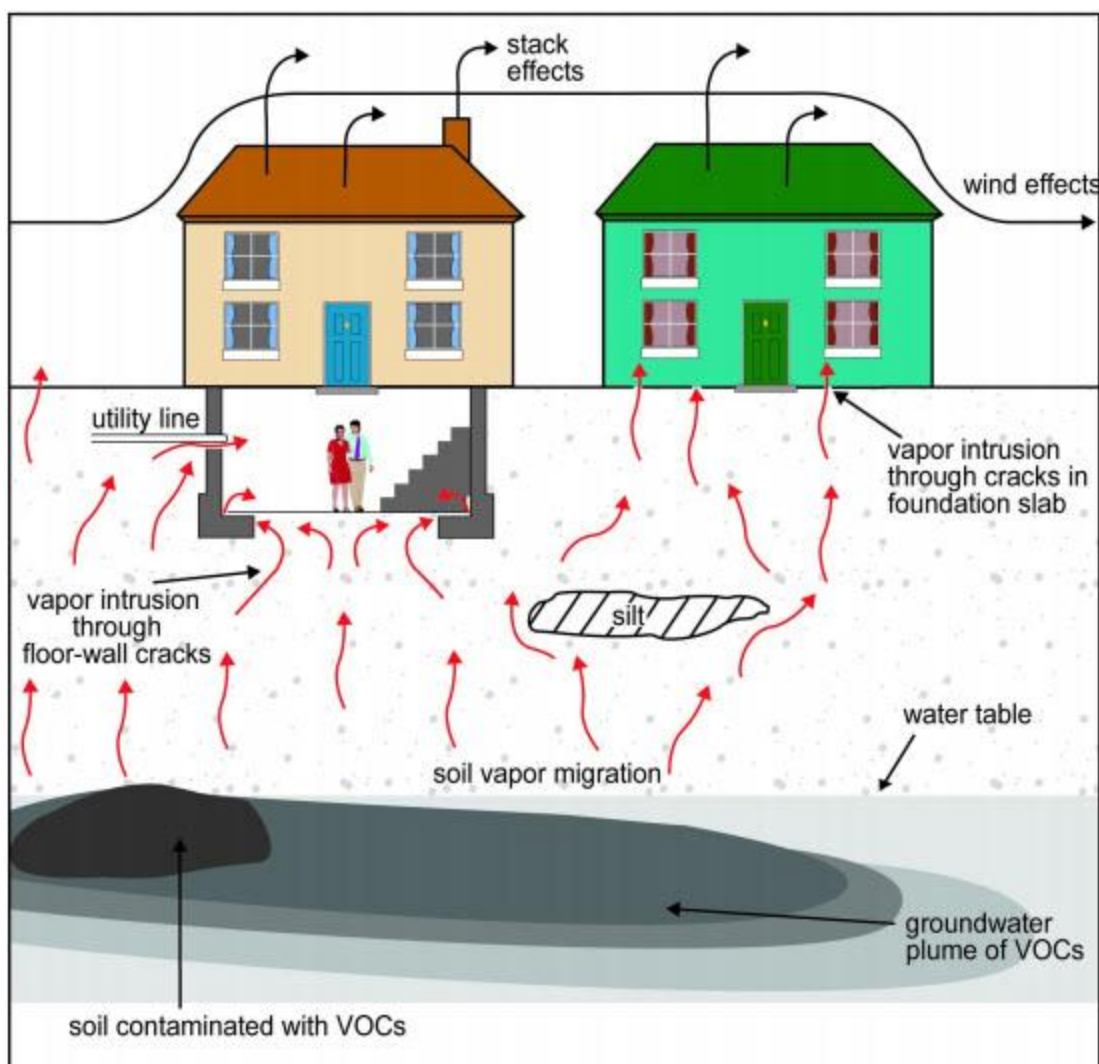


Figure 1.1: Volatile organic compounds (VOCs) like chlorinated aliphatic hydrocarbons (CAHs) can infiltrate indoor air via vapor intrusion.⁶

Although capable of aerobic transformation, PCE and TCE were originally thought to be resistant to biological degradation in anaerobic (low oxygen) environments, such as saturated groundwater aquifers. However, it was discovered in the 1980s by a series of researchers that PCE could be reduced by anaerobic bacteria to ethene via the MRD pathway illustrated in Figure 1.2.⁷⁻¹⁰ Injecting a microbial substrate such as molasses into the subsurface to induce anaerobic conditions and stimulate the growth of native bacteria that then degrade chlorinated solvent

plumes was an attractively low-cost remedial option, but early attempts often resulted in incomplete dechlorination and the accumulation of cis-DCE or VC.^{1,11} Accumulation of VC is especially problematic because it is more toxic than its parent chlorinated aliphatic hydrocarbon (CAH) compounds.¹² The Agency for Toxic Substances and Disease Registry (ATSDR) publishes a Substance Priority List that ranks contaminants by their prevalence and human health risk. In 2019, the CAH compounds PCE, TCE, cis-DCE, and VC were ranked 33, 16, 221, and 4, respectively.¹³

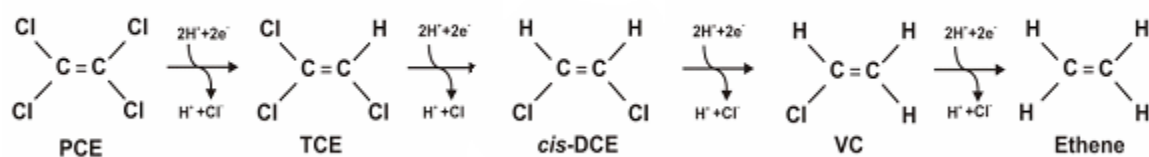


Figure 1.2: The microbial reductive dechlorination pathway, by which the toxic chlorinated aliphatic hydrocarbon (CAH) compounds PCE, TCE, *cis*-DCE and VC are degraded to harmless ethene by certain genera of bacteria.¹⁴

Later research revealed that a few different genera of bacteria are capable of dechlorinating PCE and TCE to *cis*-DCE (e.g., *Geobacter*¹⁵, *Sulfurospirillum*^{16–18}, and *Dehalobacter*¹⁹), but *Dehalococcoides* (*Dhc*) is the only genus of bacteria^{10,20} known to be capable of completely dechlorinating PCE to ethene via the MRD pathway in Figure 1.2.²¹ The vital role that *Dhc* plays in mediating complete MRD was made clear in 2001 when a survey of 24 CAH-contaminated sites found that locations with complete dechlorination occurring all had *Dhc* present, and sites where dechlorination stalled at *cis*-DCE did not have *Dhc*.²² Instead of relying on indigenous bacteria to mediate the MRD reaction (which can fail if *Dhc* is not present in adequate numbers)

the practice of injecting a bacterial inoculum (i.e., bioaugmentation) containing *Dhc* at a CAH-contaminated site became an established remediation option in the aughts.¹

The idea of bioaugmentation was not new; dozens of bacterial inocula for bioremediation were commercially available in the nineties.²³ But of the myriad of cultures available at that time, none had been demonstrated in controlled field trials to be highly effective for treatment of all CAH compounds.¹ The most extensively studied option, aerobic strains of bacteria²⁴ that could degrade TCE directly, had several flaws: (1) they degraded TCE cometabolically (i.e., as a fortuitous side effect of other metabolic processes, not as a direct means for growth), (2) they could not treat high concentrations of TCE typically present near sources of contamination, and (3) they had limited or no ability to degrade other CAH compounds.¹ The utility of *Dhc*-based inocula was first field-tested in 2001 at Dover Air Force Base in Delaware, where 35 g dry-weight of a culture containing *Dhc* injected into a hockey-rink-sized area dechlorinated 70 μM of CAH compounds into harmless ethene.²⁵

Since then, bioaugmentation inocula containing *Dhc* have been successfully utilized at hundreds of CAH-contaminated sites.¹¹ Most commercially available inocula sold for such purposes (such as BDI Plus from Regenesys, KB-1 from SiREM, SDC-9 from RNAS, and TSI-DC from Terra Systems) contain *Dhc* as well as other cultures empirically demonstrated to play a synergistic role in the MRD process.^{26–29} For example, KB-1 also contains *Geobacter* to increase the rate of PCE and TCE dechlorination, as well as fermentative bacteria that produces hydrogen gas utilized by both *Geobacter* and *Dhc* to carry out MRD.³⁰

1.2 Co-Contamination of Chlorinated Solvents with Per- and Polyfluoroalkyl Substances
Per- and polyfluoroalkyl substances (PFAS) have been used in many industries, such as nonstick cookware, stain-repellent coatings, and firefighting foam.³¹ The term PFAS encompasses a broad

family of complex organic compounds with a carbon-fluorine backbone.³² This carbon-fluorine bond is one of the strongest in organic chemistry, earning this class of compounds the moniker “forever chemicals”.³³ Once in the environment, PFAS compounds tend to degrade into a less complex category of PFAS called perfluoroalkyl acid (PFAA).³² See Figure 1.3 for a ball-and-stick diagram of an example PFAA.

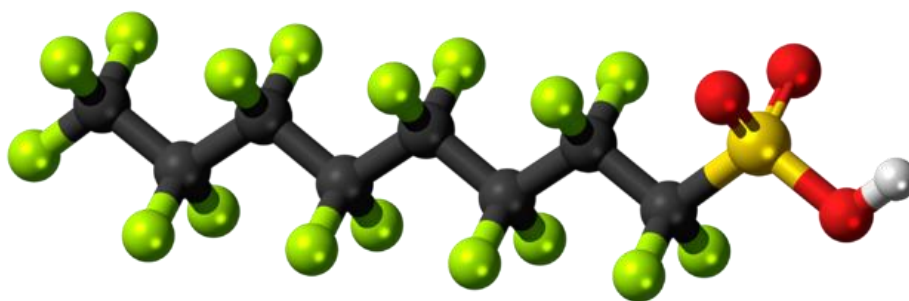


Figure 1.3: Chemical structure of the pervasive PFAS contaminant perfluorooctane sulfonate, one of the PFAA compounds that more complex PFAS tend to degrade into.³⁴ Black, green, yellow, red, and gray balls represent carbon, fluorine, sulfur, oxygen, and hydrogen, respectively.

Like chlorinated solvents, the widespread use of PFAS was followed by concerns about their effects on the human body. PFAS have been manufactured since the fifties, but their prevalence in the environment was not known until the early 2000s when analytical detection limits approached levels sensitive enough to discover the low- but ubiquitous exposure levels.³¹ They have now been found in the blood of 98% of Americans.^{35,36} Potential health effects include testicular cancer, kidney cancer, high cholesterol, decreased fertility, thyroid disease, and suppression of vaccine effectiveness.^{37,38}

The United States Air Force (USAF) currently faces a multi-billion-dollar multi-decade cleanup liability stemming from its heavy use of PFAS-based firefighting foams.^{39,40} Firefighting drills often used ignited TCE to simulate jet fuel fires, resulting in co-contamination of CAH with

PFAS at these sites because wastewater containing extinguished solvent and firefighting foam was typically discharged directly to the ground surface until the nineties.^{41,42} This comingling of PFAS and CAH contamination potentially complicates cleanup efforts. Experimental evidence suggests that firefighting foam has inhibitory effects on MRD by *Dhc*: Of three products tested in one study, the MRD of TCE was stopped altogether by two and the third product resulted in retarded dechlorination (several factors slower than a control) that ended in VC accumulation.⁴³

1.3 The Ill-Posed Inverse Problem of Estimating MRD Model Parameters

With few exceptions, papers that fit models to laboratory MRD experiments employ models based on Monod kinetics, in which the dechlorination rate of CAH compound i is⁴⁴:

$$r_i^a = \frac{k_{max,i} C_i^a X}{K_{s,i} I_i + C_i^a} \quad (1)$$

where $k_{max,i}$ is the maximum utilization rate for component i ($\text{MM}_{\text{bio}}^{-1} \text{T}^{-1}$), C_i^a is the aqueous phase concentration (ML^{-3}), X is the concentration (cells L^{-3}) of dechlorinating bacteria, and $K_{s,i}$ is the half-saturation constant for component i (ML^{-3}). The competitive inhibition factor, I_i (dimensionless), accounts for competition between the chlorinated ethenes for MRD-mediating enzymes on the bacterial cell membrane. See the next chapter for an introduction to Monod kinetics models applied to the microcosm system.

Originally adapted from the enzyme literature, Monod kinetics is an empirical model formulation for microbe-mediated processes where the reaction regime straddles first-order kinetics (i.e., doubling the reactant concentration doubles the reaction rate) and zero-order kinetics (i.e., some maximum capacity of the cell to render the reaction is exceeded, and further reactant concentration increases do not increase the reaction rate).⁴⁵ The flexibility afforded by such a “mixed-order” regime is a double-edged sword. Very close model fits to laboratory experiments

are possible, but precise parameter estimation can become extremely difficult: equally good model fits are achieved by a wide range of plausible parameter values.⁴⁶ Widely adapted model enhancements to account for competition among CAH compounds for limited MRD-mediating enzymes on the cell exterior (i.e., competitive inhibition) have layered further complexity onto an inverse problem that was already severely ill-posed. What has resulted over the last quarter century is a pronounced lack of consensus over the typical ranges for Monod kinetic parameters: laboratory MRD studies employing similar cultures and experimental conditions have estimated kinetic parameter values that regularly differ by multiple orders of magnitude.⁴⁴ The ill-posed nature of the inverse problem of estimating kinetic parameters from laboratory data has hindered adoption of Monod kinetics in the context where it has the greatest potential impact: field-scale MRD models implemented by researchers and practitioners.

Further model formulation enhancements meant to simulate how PFAS inhibits the rate of MRD will make the inverse problem even more difficult. Perhaps to avoid this complexity, the papers on this topic so far have simply forsaken modeling entirely. For example, one study conducted MRD batch reactor experiments with varying concentrations of PFAAs, but instead of fitting a Monod kinetics model to each experiment and comparing estimated parameters, the authors fit a line to a “linear portion” of the timeseries concentration data for each CAH compound, then compared the slopes.⁴⁷ (This crude quasi-graphical approach is highly susceptible to individual interpretation, and stands in stark contrast to the “principle component analysis” of phylogenetic sequencing data that forms the latter half of the paper.)

The same reticence to use Monod kinetics for modeling MRD can be seen in field-scale work. Predictive modeling tools available to the practitioner, such as Visual MODFLOW, MT3D-USGS, and BIOCHLOR, all simplify MRD as a sequential first-order process.^{48–50} A 420-page

monograph on bioaugmentation, commissioned by the US Department of Defense and meant to summarize decades of CAH compound treatment research for the practitioner, offers no advice on modeling.¹¹ It states that data from laboratory treatability tests (the same type of experiment typically modeled in the MRD literature) can be used to answer simple yes or no questions (“Is bioaugmentation necessary?”), but has no recommendations for combining that data with a model to answer quantitative questions (“How long will bioremediation take?”). The bioaugmentation inoculum manufacturer SiREM offers laboratory treatability testing as a service, but other than estimating degradation half-life times (i.e., a first-order model parameter), no modeling is generally requested by clients.⁵¹ Of the few relevant papers in the scientific literature, one attempt⁵² to use laboratory estimated Monod kinetics parameters to model field-scale MRD resulted in overprediction of *in situ* degradation rates, although other results^{53,54} suggest that such overprediction can be avoided if subsurface heterogeneity is explicitly modeled.

Model complexity is not bad if the implications of that complexity on parameter estimation reliability is quantified and accounted for. If the parameter estimation uncertainty is too great for our purposes (e.g., accurate field-scale predictions, statistical inference of two cultures or experimental conditions), then different parameter estimation techniques and experimental designs should be tested.

1.4 Objective and Organization of This Dissertation

The objective of this dissertation is to present a series of research results that, when taken together, form a set of best practices for estimating Monod kinetic parameters from laboratory batch reactor and microcosm experiments.

The chapters are as follows:

1. **Introduction**
2. **Example Estimation of Monod Parameters:** The MRD model and the experimental system it describes is introduced. A fit of this model using typical literature methods is presented.
3. **Microbial Reductive Dechlorination is Not Inhibited by Perfluoroalkyl Acids (PFAAs) under Environmentally Relevant Concentrations:** To evaluate the inhibitive effect that PFAS has on MRD, the technique of weighted least squares is used to fit the MRD model to batch reactor experiments with varying concentrations of PFAAs. As a secondary objective, improvements to mechanisms for modeling experimental mass loss are explored.
4. **Quantifying Impacts of Microcosm Mass Loss on Kinetic Constant Estimation:** A series of numerical model fitting experiments with synthetic microcosm data are conducted to evaluate how the common oversight of ignoring experimental mass loss can affect Monod parameter estimation.
5. **On the Reliable Estimation of Sequential Monod Kinetic Parameters:** Monte Carlo experiments comprised of more than a million numerical model-fitting experiments and more than one hundred thousand sets of plausible Monod parameter values are conducted to (1) evaluate the parameter estimation uncertainty that various experimental designs yield and (2) test the reliability of different parameter interval estimation algorithms.
6. **Conclusions and Recommendations for Future Work**

2 Example Estimation of Monod Parameters

2.1 Introduction

The content of this chapter is largely abstracted from a paper by Lurong Yang, Jason P. Hnatko, Jack L. Elsey, John A. Christ, Kurt D. Pennell, Natalie L. Cápiro, and Linda M. Abriola.

(Reprinted with permission from Water Research Volume 193, 2021, 116842.

<https://doi.org/10.1016/j.watres.2021.116842>) In this paper a finite difference, multi-species mass balance model was used to estimate microbial reductive dechlorination (MRD) parameters from batch microcosm data, and then those parameters were used to simulate a bioremediation experiment conducted in a laboratory-scale aquifer cell. The microcosm MRD model and its fitting results are described in the following sections to summarize work that the author personally performed for his PhD, as well as to introduce the Monod kinetics microcosm model used in subsequent chapters.

The parameter estimation approach taken in this paper is comparable to many other studies in the literature that used a gradient-based or quasi-Newton algorithm (e.g. the generalized reduced gradient optimizer embedded in Microsoft Excel⁵⁵) to minimize a least-squares objective function. Subsequent chapters describe an improved estimation approach where an implementation of the differential evolution algorithm⁵⁶ is used to vary fitting parameters such that the likelihood of the observed data is maximized.

2.2 Microcosm Batch Model

The dechlorination rate of CAH compound i given by Monod kinetics is:

$$r_i^a = \frac{k_{max,i} C_i^a X_j}{K_{s,i} I_i + C_i^a} \quad (1)$$

where $k_{max,i}$ is the maximum utilization rate for component i ($\text{MM}_{\text{bio}}^{-1}\text{T}^{-1}$), C_i^a is the aqueous phase concentration (ML^{-3}), X_j is the concentration ($\text{M}_{\text{bio}}\text{L}^{-3}$) of cells for the TCE dechlorinator ($j = \text{Geo}$) or the *cis*-DCE and VC dechlorinator ($j = \text{Dhc}$), and $K_{s,i}$ is the half-saturation constant for component i (ML^{-3}). The competitive inhibition factor, I_i (dimensionless), accounts for inhibition caused by competition between the chlorinated ethenes for MRD-mediating enzymes on the bacterial cell membrane. The overall degradation rate for each chemical compound is the sum of the relevant dechlorination rates, i.e. $R_{\text{TCE}}^a = -r_{\text{TCE}}^a$, $R_{\text{DCE}}^a = r_{\text{TCE}}^a - r_{\text{DCE}}^a$, $R_{\text{VC}}^a = r_{\text{DCE}}^a - r_{\text{VC}}^a$, and $R_{\text{ETH}}^a = r_{\text{VC}}^a$.⁵⁷

Each MRD reaction requires an electron acceptor (the CAH compound being dechlorinated) and an electron donor (typically hydrogen derived from the fermentation of lactate). Some MRD models incorporate an additional factor in Equation 1 meant to approximate rate limitation caused by electron donor scarcity, but that approach was not taken here because sodium DL-lactate was supplied in excess of what the MRD process would require.⁵⁴

The model assumes that the chemical species in the microcosm exist in three phases: as a solute in the groundwater (aqueous phase, a), as a vapor in the head space (gas phase, g), or as a sorbate on the surface of the soil particles (sorbed phase, s). Furthermore, it assumes that the mass of chemical species i in the microcosm can only change in three ways:

- Chemical species i is converted into another chemical species via dechlorination.
- Chemical species i is removed from the microcosm when samples of the aqueous phase are taken.

- Chemical species i in the gas phase escapes from the microcosm via the septum. Abiotic microcosm experiments suggested that this diffusion process could be adequately modeled as first-order, dependent on the concentration of chemical species i in the aqueous phase.

The mass balance equation for chemical species i in a microcosm is then given by:

$$\frac{d}{dt}(V^a C_i^a) + \frac{d}{dt}(V^g C_i^g) + \frac{d}{dt}(q_i M_s) = V^a R_i^a + Q^a C_i^a + k_i^{dl} C_i^a \quad (2)$$

where V^a is the volume of aqueous phase (L), C_i^a is the aqueous phase concentration in Equation 1, V^g is the volume of gas phase (L), C_i^g is the concentration of chemical species i in the gas phase (mM), q_i is the molar mass of chemical species i sorbed per mass of soil (mmol per g), M_s is the mass of soil (g), R_i^a is the transformation or production rate of chemical species i in the aqueous phase (mM/d), Q^a is the rate of aqueous phase sampling averaged over the duration of the experiment (L/d, negative value), and k_i^{dl} is a rate constant for gas escape from the microcosm (1/d, negative value). Using linear sorption parameters measured in this study and Henry's law, functions of the aqueous phase concentration C_i^a can be substituted for q_i and C_i^g in Equation 2 and the ODE for chemical species i can be rearranged to form

$$\frac{d}{dt}(C_i^a) = \frac{V^a R_i^a + \frac{Q^a C_i^a}{H_i R T} + k_i^{dl} C_i^a}{V^a + \frac{V^g}{H_i R T} + K_{d,i} M_s} \quad (3)$$

where H_i is the Henry's law constant for chemical species i (mmol/m³ Pa), R is the gas constant (J/K mmol), T is the temperature (293 K), and $K_{d,i}$ is the linear sorption constant for chemical species i .

To close the system, volume balance equations are also needed:

$$\frac{d}{dt}(V^a) = Q^a \quad (4)$$

$$\frac{d}{dt}(V^g) = -Q^a \quad (5)$$

where Q^a is a constant aqueous phase sampling rate meant to approximate aqueous phase samples being removed from the system at a regular interval.

Biomass growth for each population is modeled as a function of dechlorination rate, yield coefficient, Y_i ($M_{\text{bio}}M^{-1}$), and a first order endogenous decay coefficient, $k_{b,j}$ (T^{-1}):

$$\frac{dX_j}{dt} = \sum_i (Y_i r_i^a) - k_{b,j} X_j \quad (6)$$

where i is TCE if j is *Geo*, and i is *cis*-DCE and VC if j is *Dhc*.

The entire system of eight ordinary differential equations (ODEs), composed of Equation 6 for *Dhc* and *Geo*, Equation 3 for the VOC compounds TCE through ethene, and Equations 4 and 5 for the aqueous and gaseous phase volumes, is then solved via an explicit Runge-Kutta algorithm⁵⁸ implemented in the Scipy Integration and ODEs Module⁵⁹.

2.3 Initial Values

Integration of the system of eight ODEs requires initial values for the eight dependent variables.

The microcosm experiments to be modeled in this chapter (MC-1, MC-2, and MC-3) are comprised of 160-mL glass vessels containing water, soil, bioaugmentation inoculum, and TCE.

The microcosms also contain sodium lactate as fermentable electron donor at a concentration high enough to preclude the need^{60,61} to include fermentation in the model formulation. It is assumed that only TCE is present at time zero, and the initial aqueous TCE concentration for each microcosm (0.172, 0.147, and 0.157 mM for microcosms MC-1, MC-2, and MC-3) is

determined by averaging the total mmols of VOC compounds in the microcosm bottle based on the first five GC measurements, assuming equilibrium partitioning between the aqueous, gaseous, and sorbed phases. The initial *Geo* biomass concentration (2.6×10^{-5} mg-cell/L for all microcosms) is calculated with the volume of KB-1[®] inoculum added to the microcosms and a measurement of the *Geo* biomass concentration in that inoculum. The initial *Dhc* biomass concentration for each microcosm (2.43×10^{-3} , 4.98×10^{-3} , and 7.26×10^{-3} mg-cell/L for microcosms MC-1, MC-2, and MC-3) is set equal to the *Dhc* biomass concentration measurement made on day one. The initial aqueous and gaseous phase volumes (100 mL and 60 mL for all microcosms) were based on measured masses of the components added to each microcosm bottle.

2.4 Determining Diffusive Mass Loss Parameters

The diffusive mass loss flux of VOC compound *i* through a septum is

$$J_i = -D_i^r \frac{dC_i^r}{dl} \quad (7)$$

where D_i^r is the diffusion coefficient for compound *i* in the septum material phase *r* and *l* refers to position along a centerline passing through the septum material blocking the microcosm bottle opening.

The rate of diffusive mass loss $J_i A^r$ through the septum of cross-sectional area A^r is first-order dependent on the aqueous phase concentration C_i^a if the following assumptions hold true:

- The concentration profile of compound *i* across the septum of thickness Δl maintains a steady state such that it decreases linearly from an initial concentration of $C_{i,0}^r$ on the internal face of the septum to a concentration of zero on the external face of the septum.

- The initial concentration $C_{i,0}^r$ is linearly proportional to the aqueous phase concentration C_i^a . That is,

$$C_{i,0}^r = K_i^{ra} C_i^a \quad (8)$$

where K_i^{ra} is a constant. The value of K_i^{ra} changes depending on what phase (aqueous or gaseous) the septum is in contact with during the experiment.

Under the above assumptions, the diffusive mass loss rate becomes

$$J_i A^r = -D_i^r \frac{C_{i,0}^r - 0}{\Delta l} A^r = -A^r D_i^r \frac{K_i^{ra} C_i^a}{\Delta l} = k_i^{dl} C_i^a \quad (9)$$

where the diffusive mass loss coefficient k_i^{dl} [$L^3 T^{-1}$] is

$$k_i^{dl} = -\frac{A^r D_i^r K_i^{ra}}{\Delta l} \quad (10)$$

k_i^{dl} can be estimated with short-term abiotic microcosm experiments (i.e. when $R_i^a = 0$ and total time is less than approximately 50 days).

$$\frac{d}{dt} (C_i^a) = \frac{\frac{C_i^a Q^a}{H_i RT} + k_i^{dl} C_i^a}{V^a + \frac{V_g}{H_i RT} + K_i^d M_s} \quad (11)$$

$$k_i^{dl} \approx \left(\frac{V^a}{\overline{C_i^a}} + \frac{V^g}{\overline{C_i^a} H_i RT} + \frac{K_i^d M_s}{\overline{C_i^a}} \right) \left(\frac{\Delta C_i^a}{\Delta t} \right) - \frac{Q^a}{H_i RT} \quad (12)$$

where $\Delta C_i^a / \Delta t$ is the slope of the best fit line to the concentration data and $\overline{C_i^a}$ is the mean of all measured C_i^a values.

The diffusive mass loss coefficients for other compounds can be estimated based on the results of three abiotic microcosms with TCE (Figure 2.1) via Graham's Law:

$$k_q^{dl} = k_p^{dl} \sqrt{\frac{M_p}{M_q}} \quad (13)$$

where M_i is the molar mass of compound i , p represents one VOC compound (TCE in this context) and q represents another VOC compound.

2.5 Microcosm Dechlorination Model Fitting Procedure

Preliminary simulations demonstrated that adequate model fits could be achieved without including competitive inhibition in the model, so the competitive inhibition factors I_i are set to unity. Given the large liquid to solid ratios in the microcosms, it is assumed that all dechlorination occurred in the aqueous phase.

Data (times series of biomass and VOC concentrations) from three microcosm replicates (MC-1, MC-2 and MC-3) and from an abiotic control experiment are fit using the above described batch model. Mass loss estimates are obtained from abiotic control experiments using the procedure described above (see Figure 2.1): The diffusive loss coefficients used in the model are $k_{TCE}^{dl} = -1.87 \times 10^{-4}$ L/d, $k_{DCE}^{dl} = -2.18 \times 10^{-4}$ L/d, $k_{VC}^{dl} = -2.72 \times 10^{-4}$ L/d, and $k_{ETH}^{dl} = -4.06 \times 10^{-4}$ L/d.

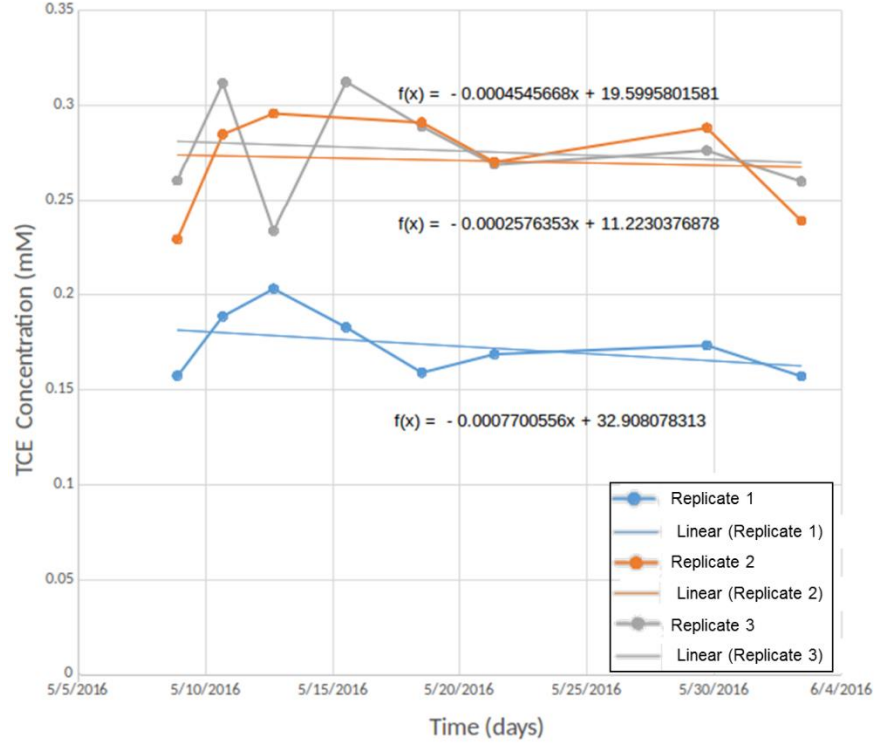


Figure 2.1: Abiotic microcosm experiments used to estimate diffusive loss coefficients

Parameter fitting is accomplished by minimizing the combined mean square error (f):

$$f = \frac{1}{n_i n_k} \sum_{i=1}^{n_i} \sum_{k=1}^{n_k} (C_{ik}^{a,measured} - C_{ik}^{a,model})^2 + \frac{\alpha}{n_b} \sum_{b=1}^{n_b} (\log X_{Dhc,b}^{a,measured} - \log X_{Dhc,b}^{a,model})^2 \quad (14)$$

where n_i is the number of VOC compounds, n_k is the number of VOC sample data per compound, C_{ik}^a is the aqueous phase concentration (mM) of VOC compound i in sample k , α is a weighting constant, n_b is the number of Dhc biomass concentration samples, and $X_{Dhc,b}^a$ is the aqueous phase Dhc biomass concentration. A nonlinear least squares optimization algorithm, “L-BFGS-B” as implemented in the SciPy Optimization and Root Finding Module^{59,62}, is employed to find the best-fit for three maximum substrate utilization rates ($k_{max,TCE}$, $k_{max,DCE}$ and $k_{max,VC}$) and two yield coefficients (Y_{DCE} and Y_{VC}). MC-2 data are fit first for all five parameters

and then the fitted MC-2 yield coefficients are held constant for subsequent maximum substrate utilization rate fits to MC-1 and MC-3 data to improve the uniqueness of fits. The *Dhc* decay rate ($k_{d,DHC}$) is estimated by fitting an exponential decay model to two qPCR measurements from the MC-1 reactor, taken after all chlorinated ethenes had undergone reductive dechlorination. *Geo*-related parameters ($k_{b,Geo}$ and Y_{TCE}) and half-saturation constants ($K_{s,i}$) are adopted from the literature (Table 2.1).

The steps undertaken to adjust the fitting parameters ($k_{max,TCE}$, $k_{max,DCE}$, $k_{max,VC}$, Y_{DCE} , and Y_{VC}) to fit the MRD model to microcosms MC-1, MC-2, and MC-3 are listed in detail below. See Table 2.1 for a list of all fitted parameter values. All model fits are performed at least five times with varying initial guesses for the fitting parameters to ensure that a global minimum for the objective function is found and that all fitting parameters were uniquely identifiable.

1. A value of Y_{TCE} is selected from the literature range (10.27 mg-cell/mmol) that when combined with the range of literature values for $k_{max,TCE}$ allows the simulated aqueous TCE concentration to drop to zero within the approximately 4 day duration observed in the microcosm experiments.
2. Measurements indicate that the *Dhc* biomass abundance reached different peak values in each of the microcosms (see Figure 2.2), with MC-1 exhibiting the greatest peak value and MC-3 exhibiting no net growth. Microcosm MC-2 exhibits the median level of growth, so chlorinated ethenes and ethene concentrations via GC and *Dhc* abundance via qPCR data from that experiment are used to find values for all five fitting parameters that minimize Equation 14 with $\alpha = 10^{-4}$. Y_{DCE} and Y_{VC} values fit in this step are then used for all three microcosms.

3. Using GC measurements of chlorinated ethene and ethene concentrations from microcosm MC-1 and MC-3 as well as the Y_{DCE} and Y_{VC} values fit in step 2, values for the three maximum substrate utilization rates ($k_{max,TCE}$, $k_{max,DCE}$, $k_{max,VC}$) are found to minimize Equation 14 with $\alpha = 0$ for microcosm MC-1 and MC-3.

2.6 Microcosm Dechlorination Model Fitting Results

In the three replicate microcosm experiments an average of 49.3 (± 4.5) days was required to transform 95% of the TCE to ethene (Figure 2.2). Fitted microcosm model simulations were generally in good agreement with measured concentrations, with combined mean squared errors (f) of 4.1×10^{-4} , 7.6×10^{-5} and 5.2×10^{-5} for the three replicates (Figure 2.2). The fitted parameters, including $k_{max,TCE}$, Y_{DCE} , Y_{VC} and $k_{b,Dhc}$ fell within the range of published values (Table 2.1), although $k_{max,DCE}$ and $k_{max,VC}$ were larger than previously reported values ($\sim 40\%$ greater for DCE and $\sim 90\%$ greater for VC). This discrepancy may be due to differences in experimental conditions (soils, substrates, growth medium, and temperature) employed here in comparison with those used in previous studies^{15,63–65} that used KB-1[®]. The variation in fitting methods (e.g., the algorithms and objective functions) and MRD model formulation (e.g., open or closed system, the form of competitive inhibition) also play an important role in determining the MRD parameters and coefficients.⁵⁷

2.7 Maximum Likelihood Estimation

The combined mean square error (Equation 14) works well as a model-fitting objective function because it balances the different magnitudes of GC and qPCR measurements, but it can only be used to determine point estimates of the parameters. If the objective of model fitting is to calculate confidence intervals for model parameters or perform other forms of statistical inference, then a maximum likelihood estimation approach should be taken. In this category of regression schemes, model residuals are weighted by measurement precision such that the global

minimum of the model-fitting objective function coincides with the global maximum of the likelihood function (i.e., the joint probability of the observed data, conditioned on the parameter values). The weighted-least-squares regression scheme described in later chapters is a simple form of maximum likelihood estimation that leverages information about measurement precision while still employing enough pragmatic statistical assumptions to keep the estimation process straightforward.

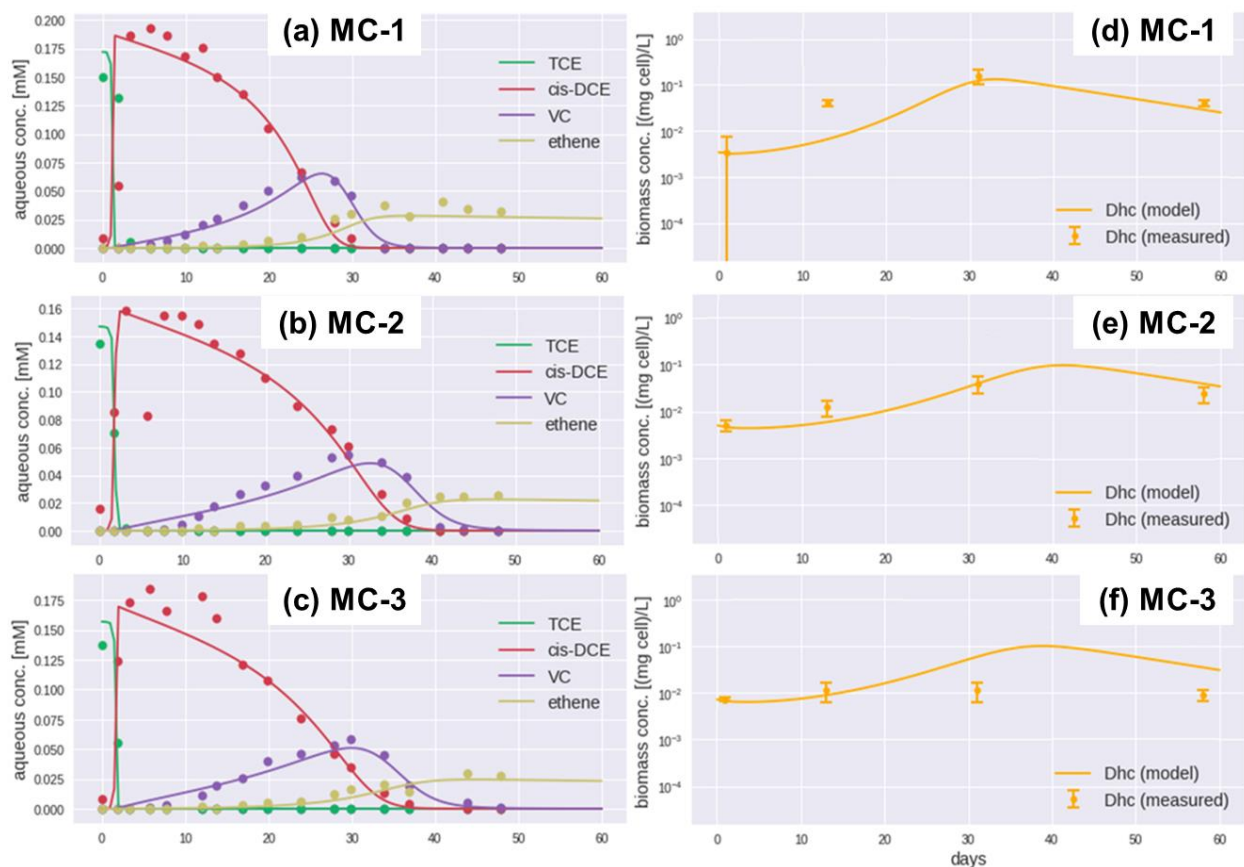


Figure 2.2: Modeled results and measured values for a set of three parallel microcosm trials (“MC-1”, “MC-2”, and “MC-3”). Figures (a)-(c) show the comparison of modeled and simulated results for aqueous phase VOC concentrations. Figures (d)-(f) show comparison for *Dhc* biomass concentrations.

Table 2.1: Microcosm Reductive Dechlorination Model Parameters

Parameter	This study	Type	Published values	References
$k_{max,i}$ ($\mu\text{mol}/(\text{mg cell-d})$) $\times 100$ ^a				
TCE	6.7 (8.5, 5.6, 6.0)	Calibrated	0.05-10.4	15,63–65
DCE	7.4 (9.3, 6.8, 6.0)	Calibrated	0.01-5.3	
VC	3.9 (4.3, 3.8, 3.5)	Calibrated	0.06-2.1	
$K_{s,i}$ (μM)				
TCE	5.0	Averaged literature value	0.1-10	66
DCE	74.5	Averaged literature value	1.6-150	
VC	50.4	Averaged literature value	1.1-100	
Y_i (mg cell/mmol) ^b				
TCE	10.27	Averaged literature values	0.14-78	15,63–66
DCE	0.11	Calibrated	0.04-20	
VC	0.96	Calibrated	0.40-20	
$k_{b,j}$ (1/day) ^c				
<i>Geo</i>	0.05	Commonly used literature value	0.05	67
<i>Dhc</i>	0.066 (0.031-0.066)	Calibrated	0.05	

^a Fitted to the microcosm experiments. Averaged values and best-fit values for three trials (bracketed) are listed in order (i.e., MC-1, MC-2, MC-3).

^b Y_{TCE} was taken from the literature range. Y_{DCE} and Y_{VC} were estimated by model fitting. It was assumed that *Dhc* cell had a mass of 3.93×10^{-11} mg.⁶⁸

^c $k_{b,Geo}$ was from Christ and Abriola (2007). $k_{b,Dhc}$ was fit to measurements of biomass in the microcosm experiments; 95% confidence interval in brackets.

3 Microbial Reductive Dechlorination is Not Inhibited by Perfluoroalkyl Acids (PFAAs) under Environmentally Relevant Concentrations

The content of this chapter is derived from a paper by Jason P. Hnatko, Chen Liu, Jack L. Elsey, Sheng Dong, John D. Fortner, Kurt D. Pennell, Linda M. Abriola, and Natalie L. Cápiro. This paper has been submitted to Environmental Science & Technology for review. The author conducted all modeling tasks discussed in the following sections.

3.1 Abstract

Perfluoroalkyl acids (PFAAs) have been previously shown to inhibit microbial reductive dechlorination (MRD) of chlorinated ethene solvents. The potential negative impacts of PFAAs on microbial species performing MRD, particularly *Dehalococcoides mccartyi* (*Dhc*), and the efficacy of *in situ* bioremediation is a critical concern for comingled PFAA-chlorinated ethene plumes. Batch reactor experiments, containing a PFAA mixture and bioaugmented with KB-1®, were completed to assess the impact of PFAAs on MRD. PFAAs delayed full biodegradation of *cis*-1,2-dichloroethene (*cis*-DCE) to ethene in batch reactors. Maximum substrate utilization rates MRD were fit to batch reactor experiments using a numerical model that accounted for chlorinated ethene losses to septa. Fitted values for *cis*-DCE and vinyl chloride MRD were significantly lower ($p < 0.05$) in batch reactors containing ≥ 50 mg/L PFAAs.

3.2 Introduction

In situ bioremediation (ISB) of chlorinated solvents, including the ubiquitous groundwater contaminants tetrachloroethene (PCE) and trichloroethene (TCE), is an effective remedy implemented at numerous sites.^{69–73} Chlorinated solvents, however, rarely occur in isolation and co-contaminants can negatively impact bioremediation.^{74–77} Per- and polyfluoroalkyl substances (PFAS) are a class of emerging contaminants that are constituents of a variety of products, including firefighting aqueous film forming foams (AFFFs).^{78–80} AFFFs have been used to

extinguish fires fed by waste fuels and solvents, leading to co-release of PCE, TCE, and PFAS to soil and groundwater.^{81,82} Groundwater plumes associated with AFFF use have documented source area total PFAS concentrations as high as 7.09 mg/L.^{83–86}

PFAS encompass a large class of compounds including more than 4,000 chemicals registered with the Chemical Abstracts Service.⁸⁷ Many degrade in the environment to fully-fluorinated perfluoroalkyl acids (PFAAs), also known as terminal PFAS.^{88–93} There are no well-studied or documented biotransformation pathways that mineralize PFAAs to non-toxic endpoints, although recent studies have identified potential microbial defluorination.^{94–97} PFAAs may affect the bioremediation of other compounds, including chlorinated solvents.⁹⁸ reported inhibition of TCE degradation by *Dehalococcoides mccartyi* (*Dhc*) at PFAA concentrations above 66 mg/L in a methanogenic PCE-to-ethene dechlorinating culture. Another study found that TCE dechlorination by *Dhc* was inhibited by Ansul and National AFFFs, but not by 3M AFFF, for total PFAS concentrations ranging from 30 to 120 mg/L.⁹⁹ Thus to treat chlorinated solvents in co-mingled plumes, the conditions under which *Dhc* activity is inhibited by PFAS must be better understood.

A series of batch reactor experiments was completed to provide insight into the conditions under which PFAAs may inhibit MRD. All reactors were prepared with a mixture of ten common PFAA co-contaminants with total concentrations ranging from 0 to 120 mg/L and subsets were bioaugmented with KB-1[®] (SIREM; Guelph, ON).^{100,101} Six PFAA concentrations were tested. To provide additional quantitative and statistical comparisons among batch reactors, a numerical model was developed and employed to fit kinetic parameters including substrate utilization rates and yield coefficients.

3.3 Materials and Methods

3.3.1 Batch Reactor Configurations

The batch reactor liquid phase consisted of an anoxic growth medium¹⁰², modified to reduce total ionic strength from 102.7 mM to 19.9 mM to prevent PFOS precipitation (PFOS solubility is affected by salt concentration¹⁰³). Reducing conditions were maintained using sodium sulfide nonahydrate and L-cysteine. All growth medium was prepared with 5 mM sodium lactate as the fermentable electron donor substrate and carbon source.

PFAAs were obtained from Sigma Aldrich (St. Louis, MO) as nonafluorobutane-1-sulfonic acid (PFBS) 97%, heptafluorobutyric acid (PFBA) 98%, perfluoropentanoic acid (PFPeA) 97%, tridecafluorohexane-1-sulfonic acid potassium salt (PFHxS) >98%, undecafluorohexanoic acid (PFHxA) >97%, perfluoroheptanoic acid (PFHpA) 99%, heptadecafluorooctanesulfonic acid potassium salt (PFOS) >98%, perfluorooctanoic acid (PFOA) 95%, perfluorononanoic acid (PFNA) 97%, and perfluorodecanoic acid (PFDA) 98%.

Batch reactors were bioaugmented with KB-1[®] (SiREM; Guelph, ON).^{100,101} In contrast to previous work that explored *Dhc* strain 195 MRD in the presence of an equal component mixture of 11 PFAAs,⁹⁸ this study used a commercially available dechlorinating consortium and a mixture of 10 PFAAs, at concentration ratios approximating those measured in groundwater at Loring Air Force Base's (Aroostook County, ME) fire training area (Table 3.1). To prevent PFOS concentrations from exceeding 25 mg/L (0.050 mM) at the highest mixture concentrations, its proportion of the total PFAS mass was reduced from the 44.3% observed at the site to 15-20% and the proportions of other compounds were increased (Table 3.1, columns 1 and 2). When PFOS was added to the PFAS mixture at concentrations above 25 mg/L (0.050 mM), its

measured concentration was lower than the mass added, attributed to precipitation of PFOS from the growth medium.

Table 3.1: Composition of PFAA Mixture in Batch Reactor Experiments

PFAA	% total PFAAs (by mass) in Loring AFB Overburden	Average % total PFAAs (by mass) in Batch Reactors	Average % total PFAAs (by mass) in Microcosms (initial)	Average % total PFAAs (by mass) in Microcosms (Final)
PFBS	0.43%	5.67%	4.42%	4.93%
PFBA	3.74%	6.41%	10.3%	10.9%
PFPeA	13.3%	14.9%	18.2%	18.8%
PFHxS	14.5%	17.9%	14.0%	14.6%
PFHxA	10.2%	10.7%	18.8%	18.8%
PFHpA	5.20%	6.30%	6.16%	6.57%
PFOS	44.3%	22.7%	15.1%	12.3%
PFOA	7.02%	9.33%	8.57%	9.01%
PFNA	1.13%	3.45%	3.24%	3.45%
PFDA	0.17%	2.59%	1.08%	0.66%

Experiments were performed in 160 mL glass serum bottles capped with butyl rubber stoppers and aluminum crimp caps (Chemglass Life Sciences; Vineland, NJ) and prepared at room temperature in an anaerobic chamber with a 97% N₂/3% H₂ atmosphere (Coy Laboratory Products; Grass Lake, MI). Each batch reactor contained 120 mL of aqueous volume.

PFAA stock solution with a total concentration of 101-122 mg/L was prepared by dissolving 10 PFAAs in pre-prepared medium. The stock solution was used for 6 sets of 5 batch reactors containing initial measured concentrations of 101 mg/L, 61.7 mg/L, 49.8 mg/L, 26.5 mg/L, 11.7 mg/L, and 0 mg/L total PFAAs, to achieve a range that encompassed the 66 mg/L total PFAA concentration previously shown to impact *Dhc* strain 195.⁹⁸ Reactors were amended with 15 mL

of low salts medium containing PCE and TCE to achieve average initial concentrations of 6.5 (± 0.66) mg/L (0.039 [± 0.004] mM) PCE and 24 (± 2.6) mg/L (0.18 [± 0.02] mM) TCE. For the highest PFAA concentration reactors, the PCE/TCE solution was prepared in medium containing 101 mg/L PFAAs to maintain the target PFAA concentrations. Reactors were also amended with 0.6 mL of 200x Wolin vitamin¹⁰⁴ solution to provide nutrients, including vitamin B₁₂, necessary for dechlorinating microorganisms.¹⁰⁵ In each set of 5 batch reactors, 3 were bioaugmented with 6 mL of KB-1® inoculum (SIREM; Guelph, ON), while 2 received 6 mL of low salts growth and were sterilized via autoclave as abiotic controls.

3.3.2 Batch Reactor Sampling

Reactors were sampled every 2 to 7 days. Samples were analyzed for chlorinated ethenes by gas chromatography with flame ionization detection (GC-FID), volatile fatty acid and fluoride concentrations by ion chromatography (IC), and PFAA concentrations by liquid chromatography with tandem mass spectrometry (LC/MS-MS). Additional 1.0 mL sample aliquots were centrifuged for future DNA extraction and quantitative polymerase chain reaction (qPCR) analysis.

3.3.3 Model Formulation

A numerical model, implemented in SciPy¹⁰⁶, was formulated to simulate MRD in batch reactors using the following mass balance equation^{107,108}:

$$\frac{d}{dt}(V^a C_i^a) + \frac{d}{dt}(V^g C_i^g) = V^a R_i^b + R_i^s \quad (1)$$

where i corresponds to PCE, TCE, *cis*-DCE, VC or ethene, t is time, V^a and V^g are the aqueous and gaseous phase volumes [L³], respectively, C_i^a and C_i^g are the aqueous and gaseous phase concentrations [M/L³], respectively, R_i^b is the overall MRD reaction rate [M/L³/T], and R_i^s is the

mass transfer rate [M/T] between the reactor aqueous and gaseous phases and the stopper.

Typical of other MRD models⁶⁰, it was assumed that partitioning between aqueous and gaseous phases conformed to Henry's law and MRD was governed by Monod kinetics.

Each overall MRD reaction rate R_i^b in Equation 1 is the sum of the individual reductive dechlorination rates that increase or decrease its aqueous concentration. That is, $R_{\text{PCE}}^b = -r_{\text{PCE}}$, $R_{\text{TCE}}^b = r_{\text{PCE}} - r_{\text{TCE}}$, $R_{\text{DCE}}^b = r_{\text{TCE}} - r_{\text{DCE}}$, $R_{\text{VC}}^b = r_{\text{DCE}} - r_{\text{VC}}$, and $R_{\text{ethene}}^b = r_{\text{VC}}$. When electron donor is in excess, as in the experiments modeled, the individual reductive dechlorination rates [M/L³/T] are determined by the following Monod kinetics expression:

$$r_i = \frac{k_{\max,i} X_j^a C_i^a}{K_{s,i} + C_i^a} \quad (2)$$

where $k_{\max,i}$ is the maximum substrate utilization rate [M/T/cell], X_j^a is the biomass concentration [cell/L³] of the microbe j that mediates the dechlorination, and $K_{s,i}$ is the half-saturation constant [M/L³].

Under Monod kinetics the biomass concentration of dechlorinating microbe j changes according to:

$$\frac{d}{dt}(X_j^a) = \sum_i (Y_i r_i) - k_j^b X_j^a \quad (3)$$

where the summation is over all chlorinated ethenes that microbe j reductively dechlorinates, Y_i is the yield coefficient[cell/M], and k_j^b is the endogenous decay constant [T⁻¹].

The KB-1® inoculum used contains fermenters and other anaerobic bacteria which support MRD. The fermentation processes require a carbon source and electron donor to proceed^{60,109}. In the

experiments modeled, lactate was supplied in excess, precluding the need^{60,61} to include fermentation in the model formulation.

Geo can only perform reductive dechlorination of PCE to TCE and TCE to *cis*-DCE.^{110,111} Although some *Dhc* strains in the KB-1® inoculum are capable of dechlorinating TCE and PCE^{110,111}, for this model it is assumed that *Dhc* only transforms *cis*-DCE to VC and VC to ethene. This assumption is supported by previous MRD experiments using TCE and KB-1® that have shown that measurable *Dhc* population growth occurred only during *cis*-DCE and VC degradation¹¹², and have estimated that 80% of TCE degradation by the KB-1® consortium is attributable to *Geo*¹¹¹. Thus, in Equations 2 and 3, i is PCE and TCE when j is *Geo* and i is *cis*-DCE and VC when j is *Dhc*.

To model interphase mass transfer of each compound i between the gaseous headspace and aqueous phases, equilibrium partitioning is assumed. Applying Henry's law to Equation 1 and rearranging yields an ordinary differential equation (ODE) describing changes in C_i^a with respect to time.

$$\frac{d}{dt}(V^a C_i^a) + \frac{d}{dt}\left(\frac{V^g C_i^a}{H_i RT}\right) = V^a R_i^b + R_i^s \quad (4)$$

$$\left(V^a + \frac{V^g}{H_i RT}\right) \frac{d}{dt}(C_i^a) = V^a R_i^b + R_i^s \quad (5)$$

$$\frac{d}{dt}(C_i^a) = \frac{V^a R_i^b + R_i^s}{V^a + \frac{V^g}{H_i RT}} \quad (6)$$

where H_i is the Henry's law coefficient for compound i [M/L³/P], R is the ideal gas constant [energy / temperature / M], and T is the system temperature.

Determining the concentrations C_i^a and X_j^a for all times $t > 0$ requires numerically integrating a set of seven ODEs: Equation 3 for *Geo* and *Dhc* and Equation 6 for the chlorinated ethenes and ethene. Completing that numerical integration requires a value for R_i^s , the rate at which compound i is entering or exiting the stopper.

Initial abiotic batch reactor model fitting revealed that a simple first-order diffusive mass loss term^{107,108}, R_i^s in Equation 1, underestimated the chlorinated ethene mass in reactors over time (see Figure 3.1). To avoid potential Monod kinetic parameter estimation bias, an improved method for estimating chlorinated ethene mass loss into the rubber stopper was implemented.¹⁰⁸ That rate of mass loss is approximated with an analytical solution for planar diffusion of a chemical compound i from an aqueous phase at concentration C_i^a into a semi-infinite solid rubber phase:¹¹³

$$C_i^r(x, t) = K_i^{ra} C_i^a \operatorname{erfc}\left(\frac{x}{2\sqrt{D_i t}}\right) \quad (7)$$

Here C_i^r is the molar concentration in the rubber phase [M/L³], x is distance from the aqueous-rubber interface [L], t is time, K_i^{ra} is a dimensionless rubber-aqueous linear partitioning coefficient dictating rubber phase chlorinated ethene concentrations at $x = 0$ [(rubber molarity)/(aqueous molarity)], and D_i is the diffusion coefficient of compound i in the rubber phase [L²/T]. After estimating D_i and K_i^{ra} for PCE and TCE using abiotic batch reactor data and literature values^{114,115}, correlations based on molecular size^{116,117} and the octanol-water partitioning coefficient^{118,119} were applied to estimate D_i and K_i^{ra} , respectively, for *cis*-DCE, VC, and ethene.

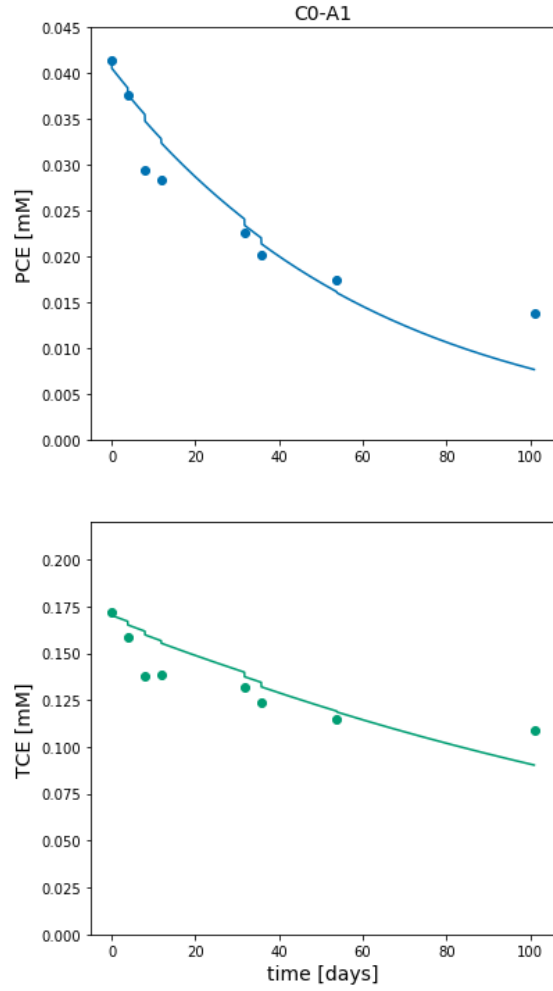


Figure 3.1: example fit of model with original mass loss formulation (curves) to abiotic batch reactor experiment data (points). See Figure 3.2 for example model fits with improved mass loss formulation.

The amount m_i^r of compound i in the stopper (idealized as a cylinder of cross-sectional area A and length L) is derived by integrating Equation 7 from $x = 0$ to $x = L$.

$$m_i^r(t) = \int_0^L A C_i^r(x, t) dx \quad (8)$$

$$m_i^r(t) = AK_i^{ra}C_i^a \left[L \operatorname{erfc} \left(\frac{L}{2\sqrt{D_i t}} \right) + \sqrt{\frac{D_i t}{\pi}} \left(1 - \exp \left[-\frac{L^2}{4D_i t} \right] \right) \right] \quad (9)$$

Which for notational simplicity will be represented below as:

$$m_i^r(t) = C_i^a f_i(t) \quad (10)$$

The analytical solution given by Equation 7 assumes a constant aqueous phase concentration C_i^a , so the expressions above cannot be used directly to model the experimental system. However, a superposition scheme based on these expressions can be implemented to approximate R_i^s , provided that the rubber phase concentration at the outside face of the stopper, $C_i^r(x = L, t)$, remains small. This superposition scheme is as follows:

Divide time into steps $k = 0, 1, 2, 3 \dots$ of length h . Assume that the rubber phase initially contains zero moles of compound i .

$$m_{i,k=0}^r = 0 \quad (11)$$

The model prediction for the total amount of compound i in the rubber phase at time step k will be a sum of k terms functionally similar to Equation 10 except C_i^a will be replaced with an aqueous concentration change $\Delta C_{i,k}^a$. The first concentration change $\Delta C_{i,k=0}^a$ is equal to the initial aqueous phase concentration.

$$\Delta C_{i,k=0}^a = C_{i,k=0}^a \quad (12)$$

After the first time step of length h the total amount of compound i in the rubber phase is approximately:

$$m_{i,k=1}^r = \Delta C_{i,k=0}^a f_i(h) \quad (13)$$

The aqueous phase concentration at time step $k = 1$ can be approximated by subtracting $m_{i,k=1}^r$ from the moles initially present in the batch reactor bottle and accounting for equilibrium partitioning between the aqueous and gaseous phases:

$$C_{i,k=1}^a = \frac{C_{i,k=0}^a \left(V^a + \frac{V^g}{H_i RT} \right) - m_{i,k=1}^r}{V^a + \frac{V^g}{H_i RT}} \quad (14)$$

where V^a is the aqueous phase volume and V^g is the gaseous headspace volume.

For all following time steps $k > 0$, the aqueous phase concentration change is:

$$\Delta C_{i,k}^a = C_{i,k}^a - C_{i,k-1}^a \quad (15)$$

The amount of compound i present in the rubber phase at the end of time step $k > 0$ is approximated with a superposition of k terms:

$$m_{i,k}^r = \sum_{n=0}^{k-1} \Delta C_{i,n}^a f_i([k-n]h) \quad (16)$$

And the corresponding aqueous phase concentration is

$$C_{i,k}^a = \frac{C_{i,k=0}^a \left(V_{k=0}^a + \frac{V_{k=0}^g}{H_i RT} \right) - m_{i,k}^r - C_{i,k-1}^a V_k^s}{(V_{k-1}^a - V_k^s) + \frac{V_{k-1}^g + V_k^s}{H_i RT}} \quad (17)$$

where V_k^s is the volume of aqueous phase that was removed for sampling during that time step k (if no sample was removed then $V_k^s = 0$).

To confirm that the rubber phase concentration on the outside face of the stopper remains small, the rubber phase concentration profile can be computed using:

$$C_{i,k}^r(x) = \sum_{n=0}^{k-1} \Delta C_{i,n}^a K_i^{ra} \operatorname{erfc}\left(\frac{x}{2\sqrt{D_i h(k-n)}}\right) \quad (18)$$

The values of C_i^a and X_j^a at the end of each time step k are determined by numerically integrating Equations 3 and 6 via an algorithm¹²⁰ available from the SciPy Integration and ODEs submodule¹⁰⁶. It is assumed that $R_i^s = 0$ over the first time step $k = 1$. For all following time steps R_i^s is approximated with a superposition that lags by one time step. Specifically, for time step $k \geq 2$ the rate of compound i entering or exiting the rubber phase is:

$$R_{i,k}^s = \frac{m_{i,k-1}^r - m_{i,k-2}^r}{h} \quad (19)$$

To quantify the empirical relationship between PFAA concentration and MRD inhibition, a log-logistic dose-response model^{112,121} was fit to the $k_{max,i}$ values. This empirical model is used to describe inhibition of microbial processes by toxic compounds where the rate of the microbial process is largely uninhibited until the concentration of the compound(s) reaches a threshold, after which the rate decreases and is formulated as:

$$k_{inh,i} = \frac{k_{max,i}}{1 + \exp\left[b_i \log\left(\frac{C_{inh}^a}{C_{50,i}^a}\right)\right]} \quad (20)$$

where $k_{inh,i}$ is the inhibited $k_{max,i}$ [M/T/cell], $k_{max,i}$ is the uninhibited $k_{max,i}$ [M/T/cell], C_{inh}^a is the aqueous concentration of the substance(s) inhibiting the microbial process (i.e., total PFAA concentration [mg/L]), $C_{50,i}^a$ is the substance(s) concentration at which the rate is halved [mg/L],

and b_i is a dimensionless parameter describing the rate of decrease around the threshold concentration (C_{50}^a).

3.3.4 Model Fitting

To obtain stopper sorption/diffusion parameters, the model described in the previous section (with $R_i^b = 0$) was first fit to abiotic batch reactor experiments for PCE and TCE. An objective function $J_{1,i}$ was defined for $i = \text{PCE and TCE}$ to measure goodness-of-fit:

$$J_{1,i} = \sum^g \sum^k (C_{ikg}^{a,\text{meas}} - C_{ikg}^{a,\text{sim}})^2 \quad (21)$$

where g is an index corresponding to each abiotic batch reactor experiment and k is the sample time step index. Note that, since no correlation between PFAA concentration and mass loss was observed, one set of mass loss parameters was estimated using all abiotic batch reactors.

Unique best-fit values for the PCE and TCE diffusive mass loss parameters could not be determined with aqueous concentration measurements alone since there is a linear correlation between the optimal values of $\log K_i^{ra}$ and $\log D_i$. Thus, independent estimates of these parameters were sought in the literature. Göbel et al. measured D_i and K_i^{ra} for PCE in butyl rubber.^{114,115} Based upon those measurements, similar values of D_{PCE} and K_{PCE}^{ra} were selected that minimized the residuals for the PCE abiotic experiments.

The Göbel et al. measurements were then combined with commonly used correlations to estimate mass loss parameters for TCE, *cis*-DCE, VC, and ethene. To estimate the rubber phase diffusion coefficients a linear regression was fit to the squared Chung diameter versus $\log D_i$.^{116,117} To estimate the rubber-aqueous linear partitioning coefficients a linear regression was fit to the log octanol-water partitioning coefficient versus $\log K_i^{ra}$.^{118,119} The resulting D_i and K_i^{ra} estimates

for *cis*-DCE, VC, and ethene were used directly. Final D_{TCE} and K_{TCE}^{ra} values for use in the model were selected so that they minimized the squared residuals computed by Equation 21 and were close to the Göbel et al.-based estimates.

Model fitting for the biotic batch reactors was completed in two phases. The objective of the first phase was to estimate a single and complete set of Monod kinetic parameters for *Geo* and *Dhc* in systems with no PFAAs. Here, the model was fit to chlorinated ethene and qPCR biomass data from three biotic control batch reactors.^{60,122} The objective of the second phase was to estimate maximum substrate utilization rates ($k_{\text{max},i}$) for systems with varying concentrations of PFAAs. This phase employed chlorinated ethene data from batch reactor experiments with total PFAA concentrations ranging from 11.7 to 101 mg/L. During the second phase of model fitting, all parameters other than the maximum substrate utilization rates were held constant to their estimated or assumed values from the first phase of model fitting. Note that GC measurements of ethene concentration were several times larger than what would be consistent with mass balance considerations, attributed to coelution with methane. Thus, GC ethene measurements were deemed unreliable and not used in model fitting.

The optimization function used for the first model-fitting phase is:

$$J_2 = \sum_{g=1}^3 \sum_{i=1}^4 \sum_{k=1}^n \frac{(C_{i,k,g}^{a,\text{meas}} - C_{i,k,g}^{a,\text{sim}})^2}{\sigma_i^2} + \sum_{g=1}^3 \sum_{k=1}^n \frac{(X_{\text{Dhc},k,g}^{a,\text{meas}} - X_{\text{Dhc},k,g}^{a,\text{sim}})^2}{\sigma_{k,g}^2} \quad (22)$$

where g indexes to one of three triplicate control batch reactor experiments, i indexes to one of four chlorinated ethenes (PCE, TCE, *cis*-DCE, and VC), and k indexes to a sampling event. The quantities σ_i^2 [(M/L³)²] and $\sigma_{k,g}^2$ [(cell/L³)²] represent the estimated variances of measurement error for GC and qPCR analysis, respectively.

The optimization function used for the second model-fitting phase is:

$$J_{3,mg} = \sum_i^i \sum_k^k \frac{(C_{i,k}^{a,\text{meas}} - C_{i,k}^{a,\text{sim}})^2}{\sigma_i^2} \quad (23)$$

where m indexes over the six total PFSA concentrations tested (0 mg/L, 11.7 mg/L, 26.5 mg/L, 49.8 mg/L, 61.7 mg/L, and 101 mg/L) and g indexes over the three triplicate batch reactor experiments conducted for each total PFSA concentration m .

The GC measurement error standard deviation in Equations 22 and 23 is estimated via:

$$\sigma_i = (\text{CV})_i \bar{C}_i^{a,\text{meas}} \quad (24)$$

where $(\text{CV})_i$ is the measurement error coefficient of variation (estimated by extracting data^{108,123} from published GC calibration curves¹²⁴ and fitting a linear regression to the sample standard deviations for each standard solution) and $\bar{C}_i^{a,\text{meas}}$ is the average measured aqueous phase concentration for each chlorinated ethene.

The qPCR measurement error standard deviation in Equation 22 was estimated with triplicate qPCR measurements of the *Dhc* 16S rRNA, *Geo* 16S rRNA, *tceA*, *vcrA*, and *bvcA* genes. A linear regression in log-log space was fit using the mean of the triplicate measurements as a predictor variable and the sample standard deviation of the triplicate measurements as a response variable. The resulting estimate of $\sigma_{k,g}$ is given by:

$$\sigma_{k,g} = 2.26 (X_{\text{Dhc},k,g}^{a,\text{meas}})^{0.891} \quad (25)$$

where $X_{\text{Dhc},k,g}^{a,\text{meas}}$ and $\sigma_{k,g}$ have units of genes/mL.

A differential evolution algorithm¹²⁵, implemented in the SciPy Optimization and Root Finding Module¹⁰⁶, was used to identify a unique set of calibration parameter values for each model fitting that minimizes the respective optimization function (Equations 22 or 23).

The optimization function used to fit the log-logistic dose-response model (Equation 20) is:

$$J_{3,i} = \sum_m \sum_g (k_{inh,img}^{est} - k_{inh,img}^{sim})^2 \quad (26)$$

where m indexes over the five non-zero total PFAA concentrations, g indexes over the three triplicate batch reactor experiments conducted for each total PFAA concentration, $k_{inh,img}^{est}$ are the estimated inhibited maximum substrate utilization rates from the second model-fitting phase, and $k_{inh,img}^{sim}$ are the inhibited maximum substrate utilization rates predicted by Equation 20. An optimization was performed to minimize Equation 26 using the Levenberg-Marquardt algorithm^{126,127} implemented the SciPy Optimization and Root Finding Module¹⁰⁶, yielding a best-fit b_i and $C_{50,i}^a$ value for each chlorinated ethene i .

3.4 Results and Discussion

3.4.1 Abiotic Controls

Example abiotic batch reactor model fits for the improved mass loss formulation used in this study are presented in Figure 3.2. Mass loss parameter values used in this study are presented in Table 3.2.

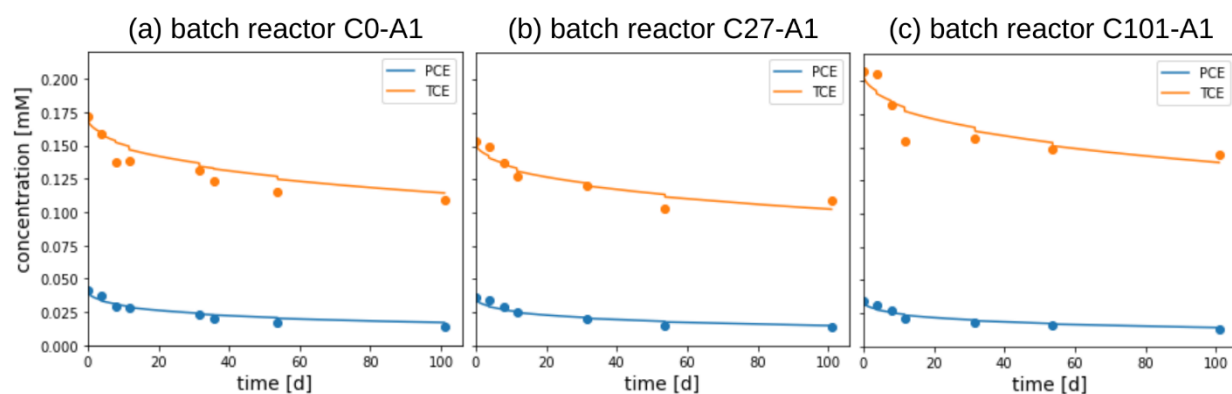


Figure 3.2: example fits of model with improved mass loss formulation (curves) to abiotic batch reactor data (points)

Table 3.2: mass loss parameters

Compound	rubber-aqueous linear partitioning coefficient K_i^{ra} [dimensionless]	rubber phase diffusion coefficient D_i [dm^2/d]
PCE	6.6×10^2	1.9×10^{-6}
TCE	1.6×10^2	3.49×10^{-6}
<i>cis</i> -DCE	8.4	2.39×10^{-6}
VC	3.3	3.09×10^{-6}
ethene	1.6	3.99×10^{-6}

3.4.2 Biotic Controls

Weighted least squares regression of the MRD model to biotic control reactors using all Monod parameters as fitting parameters reproduced the magnitudes and temporal trends of chlorinated ethene and qPCR measurements, with a McFadden pseudo R^2 value¹²⁸ of 0.951. Model parameters from this first phase of biotic model fitting are given in Table 3.3, and the corresponding model fits are depicted in Figure 3.3.

Table 3.3: Monod kinetic parameters

Parameter	Value	Note	Optimization Range†
maximum substrate utilization rates [mmol/cell/d]			
$k_{\max,\text{PCE}}$	7.75×10^{-13}	calibration parameter	7.54×10^{-13} to 1.57×10^{-10}
$k_{\max,\text{TCE}}$	2.36×10^{-12}	calibration parameter	7.54×10^{-13} to 1.57×10^{-10}
$k_{\max,\text{DCE}}$	1.66×10^{-12}	calibration parameter	3.93×10^{-14} to 2.08×10^{-11}
$k_{\max,\text{VC}}$	9.64×10^{-13}	calibration parameter	2.36×10^{-13} to 8.40×10^{-12}
half-saturation constant [mM]			
$K_{s,\text{PCE}}$	4.12×10^{-3}	calibration parameter	4.00×10^{-3} to 1.00×10^{-2}
$K_{s,\text{TCE}}$	4.07×10^{-3}	calibration parameter	4.00×10^{-3} to 1.00×10^{-2}
$K_{s,\text{DCE}}$	1.95×10^{-1}	calibration parameter	1.00×10^{-3} to 1.96×10^{-1}
$K_{s,\text{VC}}$	1.77×10^{-1}	calibration parameter	1.00×10^{-3} to 1.97×10^{-1}
yield coefficients [cell/mmol]			
Y_{PCE}	9.93×10^{10}	fixed to literature value ¹²⁹	not applicable
Y_{TCE}	9.93×10^{10}	fixed to literature value ¹²⁹	not applicable
Y_{DCE}	9.50×10^{10}	calibration parameter	1.02×10^9 to 5.19×10^{11}
Y_{VC}	2.25×10^{11}	calibration parameter	1.02×10^{10} to 5.19×10^{11}
endogenous decay constant [d^{-1}]			
k_{Geo}^b	1.00×10^{-2}	assumed equal to k_{Dhc}^b	not applicable
k_{Dhc}^b	1.00×10^{-2}	independently approximated prior to model fitting by observing decay in qPCR measurements made after complete MRD	not applicable
initial biomass concentration [cells/mL]			
$X_{\text{Geo},t=0}^a$	7.10×10^6	fixed to estimate based on dilution of KB-1® inoculum	not applicable
$X_{\text{Dhc},t=0}^a$	1.07×10^7	calibration parameter	2.38×10^6 to 1.42×10^7

† Optimization ranges for maximum substrate utilization rates, half-saturation coefficients, and yield coefficients are based on literature ranges for studies that used KB-1® inoculum.^{112,129,130} The optimization range for $X_{\text{Dhc},t=0}^a$ was a 95% confidence interval calculated using replicate qPCR measurements of KB-1® inoculum.

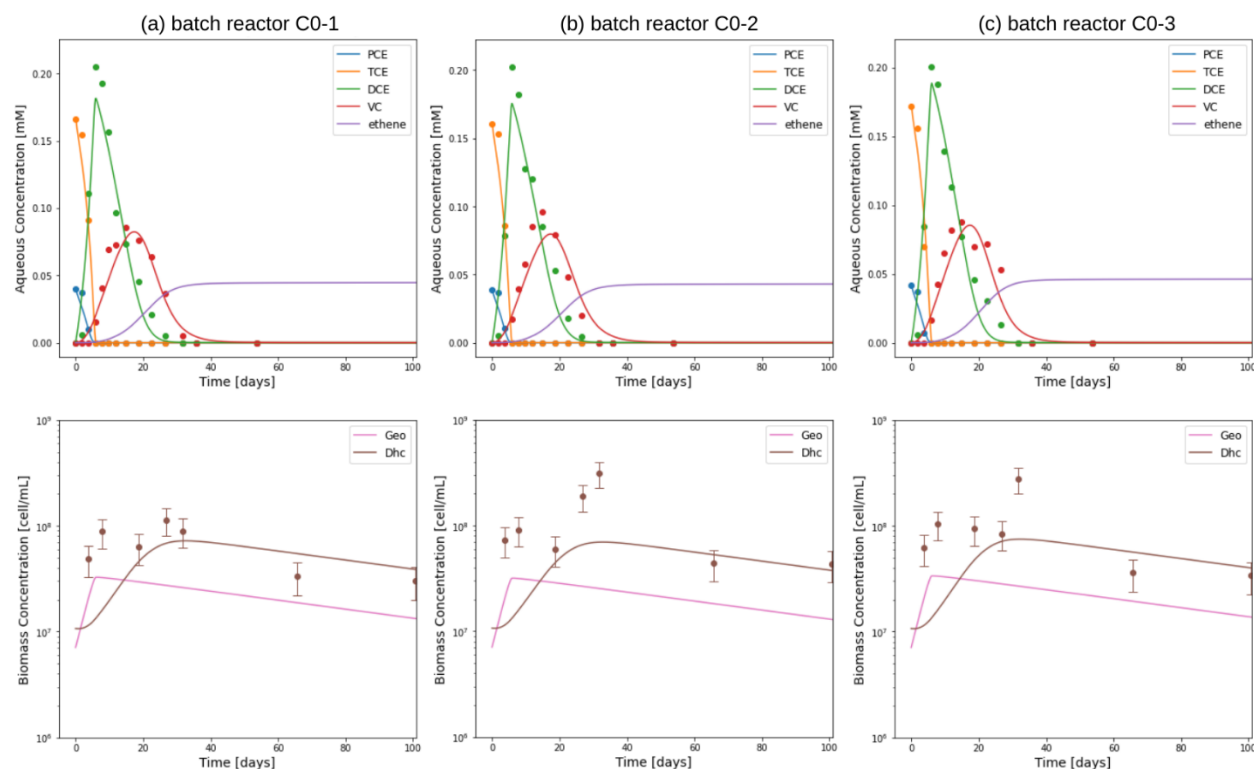


Figure 3.3: Fits to batch reactors with no PFAAs. Curves represent model values, points represent measurements, and error bars around qPCR measurements represent one standard deviation as estimated by Equation 25.

3.4.3 Batch Reactors Amended with PFAAs

In batch reactors amended with PFAAs (see Figure 3.4), the time to transform PCE and TCE to daughter products was not statistically different ($p > 0.05$) at all PFAA concentrations tested (up to 101 mg/L). The biodegradation time, i.e., time until TCE was not detected ($\text{TCE} < 0.18 \text{ mg/L}$ [0.0014 mM]), averaged $6.0 (\pm 0.0)$ days in reactors without PFAAs. In reactors with PFAAs, average biodegradation time was $5.3 (\pm 0.94)$ days, demonstrating that PFAAs did not impede biodegradation of PCE and TCE to *cis*-DCE.

MRD of *cis*-DCE to VC and VC to ethene, however, required additional time in reactors with PFAAs when compared to control reactors. In batch reactors without PFAAs, *cis*-DCE was no

longer detected (<0.15 mg/L [0.0016 mM]) after $32.0 (\pm 0.0)$ days and VC was no longer detected (<0.10 mg/L [0.0016 mM]) after $34.7 (\pm 1.9)$ days, while reactors with 11.7 mg/L PFAAs required 17% more time (37.3 ± 1.9 days) until *cis*-DCE was not detected, and 15% more time (40.0 ± 0.0 days) until VC was not detected. Similarly, the time until *cis*-DCE was not detected was 38% (44.0 ± 0.0 days), 56% (50.0 ± 2.8 days), 81% (58.0 ± 5.7 days), and 194% (94.0 ± 5.7 days) longer in reactors with 26.5 , 49.8 , 61.7 , and 101 mg/L PFAAs, respectively. The time until VC was not detected was 35% (46.7 ± 1.9 days), 44% (50.0 ± 2.8 days), 67% (58.0 ± 5.7 days), and 249% (121 ± 8.8 days) longer in reactors with 26.5 , 49.8 , 61.7 , and 101 mg/L PFAAs, respectively, when compared with reactors containing 0 mg/L PFAAs. The additional time required to fully transform *cis*-DCE to ethene in reactors with PFAAs indicates that MRD by *Dhc* was impeded by PFAAs in batch reactor experiments. MRD of PCE and TCE to *cis*-DCE was likely facilitated by *Geo*, which comprise a larger proportion of the dechlorinating community when PCE and TCE are present.^{100,131} These results suggest that *Geo* are not likely to be inhibited by PFAA concentrations that inhibit *Dhc* MRD past *cis*-DCE. There was no evidence of PFAA transformation in the batch reactors. Although measured concentrations of total PFAAs varied -13% to +0% over the course of the experiments, there was no downward trend with time and fluoride was not detected (<0.01 mM).

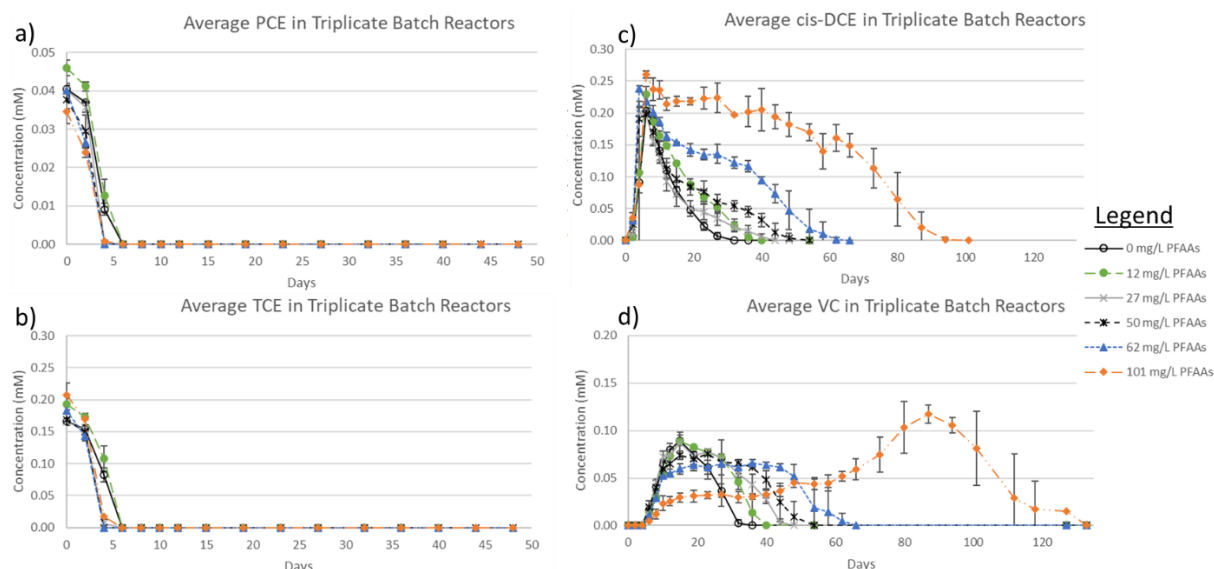


Figure 3.4: Transformation of chlorinated ethenes in batch reactor experiments. Symbols represent average **a)** PCE **b)** TCE **c)** *cis*-DCE and **d)** VC concentrations in triplicate batch reactors. Vertical error bars represent standard deviation among triplicates.

During the second phase of model fitting, the model was individually fit to each batch reactor triplicate set containing PFAAs. During the individual fitting, all Monod kinetic parameters except the maximum substrate utilization rates ($k_{max,i}$) were held constant to the biotic control best-fit values (Table 3.3). Model fits demonstrated good agreement with experimental measurements (McFadden pseudo R^2 from 0.779-0.951; see Figure 3.5), despite the lower number of fitting parameters (four $k_{max,i}$ only). This fitting yielded multiple $k_{max,i}$ estimates for each PFAA concentration tested, enabling statistical inference regarding the PFAA concentrations at which MRD is significantly inhibited. A one-sided student's t-test was used to determine if the mean $k_{max,i}$ of reactors with a certain PFAA concentration was equal to (null hypothesis) or less than (alternative hypothesis) the mean $k_{max,i}$ of reactors without PFAAs. Average fitted $k_{max,i}$ values for reactors amended with various PFAA concentrations are presented in Table 3.4.

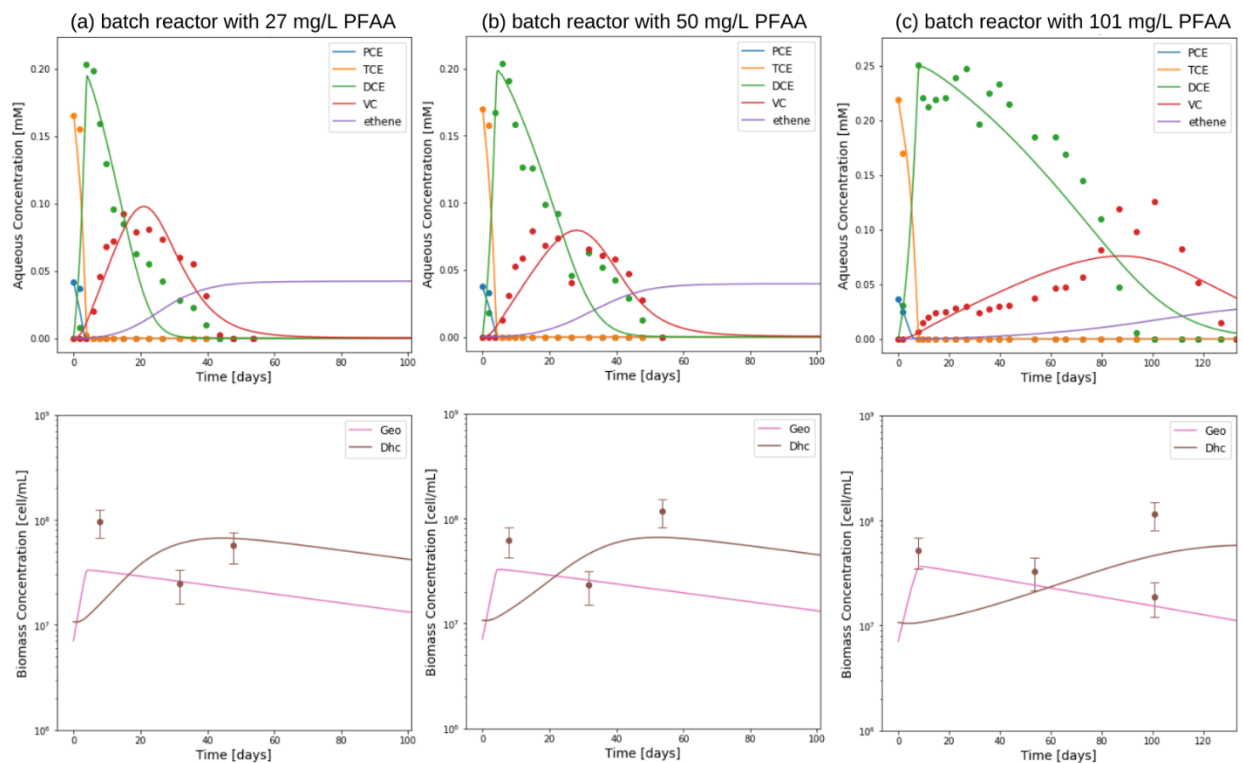


Figure 3.5: Example model fits to batch reactors with varying concentrations of PFAAs. Curves represent model values, points represent measurements, and error bars around qPCR measurements represent one standard deviation as estimated by Equation 25.

Table 3.4: Average maximum substrate utilization rates for each set of batch reactors in units of mmol/day/cell. **Shaded** entries are significantly lower than the average for the 0 mg/L reactor set ($p < 0.05$)

Batch Reactor PFAA concentration	mean $k_{max,PCE}$ ($\pm$ st.dev)	mean $k_{max,TCE}$ ($\pm$ st.dev)	mean $k_{max,DCE}$ ($\pm$ st.dev)	mean $k_{max,VC}$ ($\pm$ st.dev)
0 mg/L	7.75×10^{-13} ($\pm 1.64 \times 10^{-14}$)	2.37×10^{-12} ($\pm 6.13 \times 10^{-14}$)	1.67×10^{-12} ($\pm 9.17 \times 10^{-15}$)	9.21×10^{-13} ($\pm 8.75 \times 10^{-14}$)
12 mg/L	7.41×10^{-13} ($\pm 4.98 \times 10^{-14}$)	2.59×10^{-12} ($\pm 4.78 \times 10^{-14}$)	1.30×10^{-12} ($\pm 5.06 \times 10^{-14}$)	7.79×10^{-13} ($\pm 4.06 \times 10^{-14}$)
27 mg/L	1.09×10^{-12} ($\pm 1.66 \times 10^{-13}$)	3.45×10^{-12} ($\pm 7.67 \times 10^{-14}$)	1.55×10^{-12} ($\pm 1.70 \times 10^{-13}$)	6.02×10^{-13} ($\pm 2.12 \times 10^{-14}$)
50 mg/L	3.77×10^{-12} ($\pm 5.07 \times 10^{-12}$)	5.38×10^{-11} ($\pm 8.72 \times 10^{-11}$)	1.10×10^{-12} ($\pm 2.13 \times 10^{-13}$)	5.74×10^{-13} ($\pm 2.28 \times 10^{-14}$)
62 mg/L	7.06×10^{-13} ($\pm 4.94 \times 10^{-13}$)	5.48×10^{-11} ($\pm 8.84 \times 10^{-11}$)	5.70×10^{-13} ($\pm 6.11 \times 10^{-14}$)	5.50×10^{-13} ($\pm 1.77 \times 10^{-13}$)
101 mg/L	5.65×10^{-12} ($\pm 8.57 \times 10^{-12}$)	2.64×10^{-12} ($\pm 9.40 \times 10^{-13}$)	3.32×10^{-13} ($\pm 1.57 \times 10^{-14}$)	2.89×10^{-13} ($\pm 1.66 \times 10^{-14}$)

The average fitted $k_{max,PCE}$ and $k_{max,TCE}$ values for reactors containing PFAAs were not statistically different from the fitted $k_{max,PCE}$ and $k_{max,TCE}$ values for those without PFAAs ($p > 0.05$), demonstrating that PFAAs did not reduce (inhibit) PCE and TCE biodegradation rates for all PFAA concentrations tested (≤ 101 mg/L). Average fitted $k_{max,DCE}$ values (representing *cis*-DCE biodegradation), however, were significantly smaller ($p < 0.05$) in reactor sets with PFAA concentrations ≥ 49.8 mg/L, when compared with the average $k_{max,DCE}$ value for reactors without PFAAs. The average fitted $k_{max,DCE}$ for the 11.7 mg/L PFAA reactor set was also significantly ($p < 0.05$) lower, but the rate fit to the 26.5 mg/L PFAA reactor set was not ($p > 0.05$). These differences suggest that *cis*-DCE to VC biodegradation was inhibited at PFAA concentrations of 49.8 mg/L and higher but may or may not be inhibited at PFAA concentrations of 11.7 and 26.5 mg/L. The average $k_{max,VC}$ values (representing VC biodegradation) were

significantly lower ($p < 0.05$) when fit to reactor sets containing PFAAs at all concentrations tested when compared to fits to the reactor set without PFAAs, indicating that VC to ethene biodegradation is inhibited at total PFAA concentrations ≥ 11.7 mg/L.

The relationship between $k_{max,DCE}$ and $k_{max,VC}$ values and PFAA concentration was well-captured by the log-logistic dose-response model (Equation 20). McFadden pseudo R^2 values were 0.801 and 0.728, respectively; see Figure 3.6. Best-fit values for $C_{50,i}^a$ were 57.0 and 60.0 mg/L for *cis*-DCE and VC degradation, respectively. The best-fit values for b_{DCE} and b_{VC} were 6.78 and 2.15, respectively. These parameter values indicate that *cis*-DCE and VC biodegradation are both inhibited at similar PFAA concentrations, but the impact on the *cis*-DCE biodegradation rate is greater than on the VC biodegradation rate at a specific PFAA concentration.

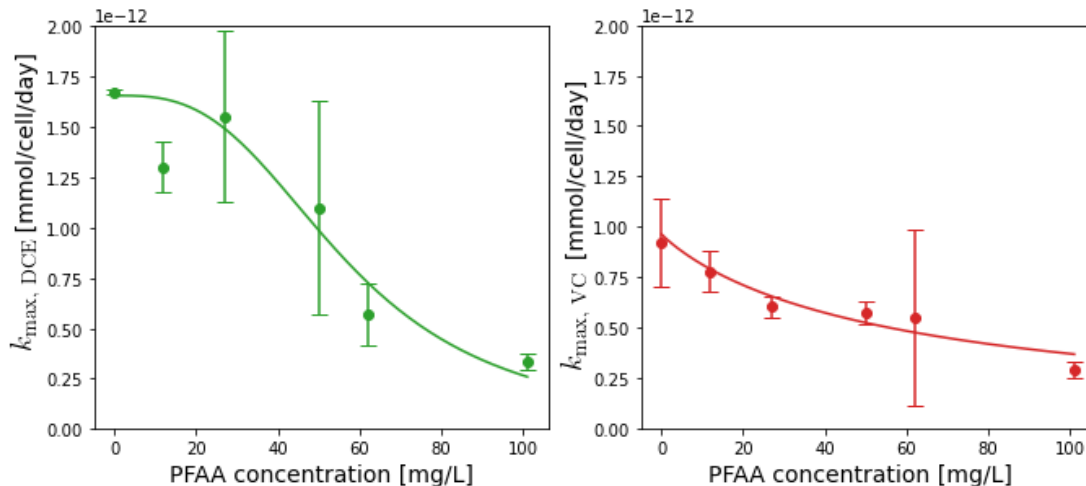


Figure 3.6: Estimated maximum substrate utilization rates for batch reactor experiments conducted with varying concentrations of total PFAAs. Plotted curves show fit of log-logistic dose-response model for inhibition of MRD by PFAA. Points represent the mean of three k_{max} estimates and each error bar indicates the corresponding 95% confidence interval.

3.4.4 Environmental Implications

Total *Dhc* growth, measured via qPCR of the *Dhc* 16S rRNA gene, was quantified for batch reactor experiments in the presence and absence of PFAAs. Average *Dhc* abundance increased from 7.59×10^6 ($\pm 1.73 \times 10^5$) gene copies/mL initially to 1.94×10^8 ($\pm 6.92 \times 10^7$) gene copies/mL at the time when all chlorinated ethenes had been transformed to ethene (day 32-101 depending on PFAA concentration). *Dhc* abundance increased 17-, 39-, 15-, 18-, 24-, and 19-fold in reactors containing 0, 11.7, 26.5, 49.8, 61.7, and 101 mg/L PFAAs, respectively. These data suggest that total *Dhc* growth and abundance should be unaffected by the presence of PFAA at bioremediation projects where the subsurface has been bioaugmented with *Dhc*-based inoculums.

In contrast, results presented above suggest that the rate of MRD can be expected to decrease if PFAA is present. However, results from a set of microcosm experiments (similar to the aforementioned batch reactor experiments except that soil was added) did not exhibit MRD inhibition at PFAA concentrations ≤ 54 mg/L.¹³² The presence of a solid phase has been shown to prevent inhibition of MRD at high PCE and TCE concentrations by providing microenvironments where microbes do not experience the high concentrations present in the bulk liquid.¹³³ The presence of certain solids also provided protection at low pH (≤ 5.5), allowing MRD to proceed at a pH that inhibited MRD when solids were absent.¹³⁴ Similarly, it is hypothesized that the PFAA inhibition of MRD observed in batch reactors may not occur at the same PFAA concentrations in the subsurface environment where a solid phase is present.

3.5 Acknowledgements

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4 Quantifying Impacts of Microcosm Mass Loss on Kinetic Constant Estimation

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The author conducted all modeling and a majority of the analysis for this paper.

4.1 Abstract

Microcosm experiments to assess microbial reductive dechlorination of chlorinated aliphatic hydrocarbons typically experience 5-50% mass loss due to frequent sampling events and diffusion through septa. A literature review, however, reveals that models fit to such experiments for kinetic constant estimation have generally failed to account for experimental mass loss. To investigate possible resultant bias in best-fit parameters, a series of numerical experiments was conducted in which Monod kinetic models with and without mass loss were fit to more than 1300 synthetic datasets, generated using published microcosm data. Models that failed to account for mass loss resulted in significant fitted parameter bias. Bias ranged from 5-45% of the parameter magnitude for Monte Carlo simulations with low (approximately 10%) mass loss to 20-120% for simulations with high (approximately 40%) mass loss. In addition, for high mass loss simulations, best-fit values consistently fell along the bounds of the optimization range. These results suggest that failure to properly account for mass loss in microcosms may lead to inaccurate estimation of kinetic constants and may explain some of the literature-reported variability in these parameters. A model is presented that provides a method for including sampling and diffusional mass losses to improve kinetic constant estimation accuracy.

4.2 Introduction

In situ bioremediation (ISB) has proven to be a cost-effective remedial alternative for sites with groundwater contaminated by chlorinated aliphatic hydrocarbon (CAH) compounds such as perchloroethylene (PCE) and trichloroethylene (TCE).¹³⁵ ISB of CAH compounds is primarily achieved via microbial reductive dechlorination (MRD), a process where the metabolic activity of naturally occurring or bioaugmented anaerobic bacteria result in the sequential transformation of PCE and TCE to cis-1,2-dichloroethylene (cis-DCE), vinyl chloride (VC), and ethene.¹³⁶ Before full-scale field application, the potential efficacy of ISB is often evaluated in laboratory microcosm experiments, where groundwater, CAH compounds, and microbes are sealed, with or without soil, in a small (typically 160 mL) glass bottle with an anoxic headspace. Periodically, small samples of the aqueous or gaseous phases are extracted through the synthetic rubber stopper (septum) and analyzed for aliphatic hydrocarbons by gas chromatography (GC). MRD models can then be fit to these microcosm data to estimate kinetic constants.

Microcosm-fitted parameters are used in practice to investigate and compare the performance of different bioremediation cultures^{137–139}. The development of new bioremediation cultures is a field of research that has attracted more than \$40 million of investment over the past year.¹⁴⁰ Some field-scale simulations, however, have overpredicted *in situ* degradation rates when laboratory-estimated biokinetic constants were employed.^{141,142} These observations may be partly attributed to formation heterogeneity at larger scales;^{143,144} recent work has demonstrated that application of rate coefficients from batch experiments can predict observed transformation data at larger scales, if heterogeneity is explicitly modeled.¹⁴⁵

Mass loss of aliphatic hydrocarbons from the microcosm inevitably results when the aqueous or gas phase is sampled. Some studies^{143,146} have demonstrated that total experimental mass loss can

exceed that caused by sampling alone, suggesting that other mechanisms contribute to mass loss. This additional loss may be attributable to diffusion of aliphatic hydrocarbons into the septum. Septa are typically constructed of butyl rubber, a material demonstrated to be permeable to hydrocarbons¹⁴⁷ (including PCE^{148,149}). Despite the likelihood of mass loss, however, a comprehensive review¹⁵⁰ of microcosm MRD models did not identify model formulations that account for experimental mass loss, suggesting that many modeling studies have assumed a closed system.

The inverse problem of fitting MRD models to microcosm data is challenging due to the large number of model parameters¹⁵⁰, high correlation between some of these parameters^{151–153}, and low measurement accuracy of biomass concentrations¹⁵⁴. Neglecting mass loss in model formulations increases uncertainty and could limit the applicability of best-fit kinetic constants. Further, the nonlinearity of MRD models implies that uncertainties and errors will influence estimated parameters in ways not easily predicted.¹⁵⁵

The goals of this paper are to (1) assess the extent of mass loss commonly encountered in previously published MRD microcosm experiments; (2) explore potential bias in best-fit kinetic parameters when mass loss is neglected in the model governing equations; and (3) provide a methodology for including mass losses in future modeling studies. The parameter bias analysis presented herein is based upon data from a series of microcosm studies¹⁴³ for the MRD of TCE and its transformation products. These experiments employed a selected microbial inoculum and a microcosm system representative of field conditions at a chlorinated solvent ISB pilot test site¹⁵⁶.

4.3 Literature Review Summary

A survey and analysis of published CAH MRD microcosm studies was undertaken to explore the range of observed experimental mass losses and to examine whether associated microcosm models considered mass loss when estimating kinetic parameters. Table 4.1 summarizes the results of this analysis. More details, including a summary of microcosm sampling methodologies, are in Section 4.6. In general, reported and estimated experimental mass losses were substantial and highly variable among the reviewed studies, averaging 23% of the initial CAH mass with a standard deviation of 18%.

Models implemented in the reviewed studies assumed equilibrium partitioning between the microcosm phases: aqueous, gaseous, and solid (if soil was present). Examination of column 6 of Table 4.1 reveals that all but three of the reviewed modeling studies appear to have also assumed that the microcosm was a closed system. A closed-system assumption, however, was often inconsistent with experimental observations. For example, Friis et al.¹⁴⁶ states that “total mass loss from the microcosms during the experiment was $41\pm7\%$ where $21\pm5\%$ was removed in aqueous and gaseous samples”. The model they fit, however, did not appear to include any mechanism for mass loss. Examination of their model predictions (extracted with WebPlotDigitizer¹⁵⁷) confirmed that total moles of microcosm aliphatic hydrocarbons did not decrease with time, confirming that the model assumed a closed system.

One of the first CAH MRD models in the literature¹⁵¹ included a mass loss mechanism (first row, Table 4.1). When a subsequent version of the model was published (second row, Table 4.1), however, it employed a simplified spreadsheet-based fitting technique in which mass losses were neglected¹⁵⁸. These authors verified that the simplified model was a valid approximation for their experimental system, but their 2-liter microcosms were much larger than those used in later studies

(generally 160 mL or smaller). This model with its simplifying assumptions has become popular for fitting microcosm experiments (despite the limitations made clear by the authors in the original work) and seems to have led to the common modeling assumption that experimental mass loss may be neglected.

Large experimental mass loss does not preclude using data from a microcosm experiment to estimate microbial kinetic parameters. However, if the model fit to such experimental data does not account for mass loss, the accuracy of fitted model parameters may be impacted. When fitting a closed-system model, the optimization algorithm will attempt to account for changes in system composition due to sampling or vessel losses (e.g. adsorption to internal microcosm surfaces, diffusion into the septum) by adjusting MRD parameters away from the values that accurately describe system kinetics.

The epistemic error introduced by ignoring experimental mass loss also violates key assumptions of the methods commonly employed in these studies to evaluate the uncertainty of estimated model parameters. For example, classical nonlinear regression analysis techniques, such as the popular spreadsheet-based method¹⁵⁸ employed by five of the MRD modeling studies in Table 4.1^{137,159–162}, hinge upon an assumption of zero-mean error to calculate interval estimates of model parameters.¹⁵⁵ That assumption is violated if large-time model predictions of aqueous concentrations are consistently higher than measured values. Other parameter estimation techniques that employ computationally intensive Monte Carlo methods (e.g. the SCEM-UA algorithm¹⁶³ used by Bælum et al.¹⁶⁴) also rely on an assumption of zero-mean error.

Based on this literature analysis, oversight of experimental mass loss seems to be common in the microcosm modeling literature. The following sections describe numerical experiments

undertaken to demonstrate how this oversight can bias best-fit kinetic parameters. A microcosm MRD model is first presented that accounts for aliphatic hydrocarbon mass loss. This model is then fit to previously published¹⁴³ microcosm data, yielding a representative set of Monod kinetic parameter values. These parameter values are used to generate synthetic GC measurements for more than a thousand hypothetical microcosm experiments with mass losses consistent with published experiments. Two models are then fit to each dataset in a Monte Carlo experiment: (1) a model that includes mass loss and (2) a closed-system model. The resulting two model fits for each synthetic dataset are compared to ascertain which model yields parameter estimates closest to the original “true” parameter values. The same overall procedure is then repeated for twenty alternate sets of “true” model parameters to confirm that experimental results are not an artifact of the particular “true” parameters selected.

Table 4.1: Measured and Modeled MRD Microcosm Mass Loss in the Literature

Reference Cited	Description	Soil Present	Experimental Mass Loss* (% of initial CAH mass)	Fit MRD Model	Model Includes Mass Loss [§]
Smith ¹⁵¹	TCE cometabolism by a methane oxidizing mixed culture	no	9% (†)	yes	yes
Smith ¹⁵⁸	same experiments as above, but fit model via simplified spreadsheet-based method that assumes no mass loss or endogenous decay	no	9% (†)	yes	no
Cupples ¹⁵⁹	VC MRD by culture “VS”	no	1-27% (‡)	yes	no
Cupples ¹³⁷	PCE, TCE, cis-DCE, and VC MRD by four different cultures	no	11% (†)	yes	no
Cupples ¹⁶⁰	cis-DCE and VC MRD by culture “VS”	no	4.5% (†)	yes	no
Yu ¹³⁹	PCE MRD by cultures “PM” and “EV”	no	≈ 0% (†)	yes	no
He ¹⁶⁵	PCE, TCE, and cis-DCE MRD by culture “FL2”	no	≈ 0% (†)	no	not applicable
Yang, Y. ¹⁶⁶	PCE and cis-DCE MRD by a culture originating from a PCE-contaminated site	no	20% (†)	no	not applicable
Yu ¹⁶⁷	PCE, TCE, and VC MRD by cultures “PM” and “EV”	no	indeterminate	yes	no

Reference Cited	Description	Soil Present	Experimental Mass Loss* (% of initial CAH mass)	Fit MRD Model	Model Includes Mass Loss [§]
Amos ¹³⁸	microbially enhanced dissolution of PCE by four different cultures	no	1-20% (‡)	yes	yes
Friis ¹⁴⁶	TCE MRD by culture “KB-1”	no	34-48% (‡)	yes	no
Friis ¹⁶⁸	TCE MRD by culture “KB-1”	yes	30% (†)	yes	no
Huang ¹⁶¹	PCE and TCE MRD by cultures “PCE1” and “BB1”	no	8.6% (†)	yes	no
Schaefer ¹⁶²	TCE MRD by culture “SDC-9”	yes	17% (†)	yes	no
Cichocka ¹⁶⁹	PCE, TCE, cis-DCE, and VC MRD by culture “BTF08”	no	25-70% (‡)	no	not applicable
Haest ¹⁷⁰	TCE MRD by culture “KB-1”	no	9% (†)	yes	no
Sabalowsky ¹⁷¹	TCE MRD by cultures “Evanite” and “Point Mugu”	no	indeterminate	yes	no
Malaguerra ¹⁷²	TCE MRD by a culture originating from a PCE-contaminated site	yes	28% (†)	yes	no
Harkness ¹⁷³	TCE MRD by culture “KB-1”	yes	indeterminate	yes	no
Bælum ¹⁶⁴	TCE MRD by culture “KB-1”	yes	45% (†)	yes	no
Schneidewind ¹⁷⁴	TCE MRD by a culture originating from a TCE-contaminated site	yes	indeterminate	yes	no
Yang, L. ¹⁴³	TCE MRD by culture “KB-1”	yes	14% (‡)	yes	yes

Notes:

* Percentages given indicate the portion of the total moles of aliphatic hydrocarbons (PCE, TCE, cis-DCE, VC, and ethene) that left the microcosm due to sampling, diffusion into a septum plug, or other mass loss mechanism over the course of the experiment. For studies where the extent of mass loss is labeled as “indeterminate”, mass loss is neither quantified in the text nor can it be estimated from plotted data.

† The indicated experimental mass loss was determined by extracting measurement data from a figure with WebPlotDigitizer¹⁵⁷.

‡ The indicated experimental mass loss was stated by the authors of the cited study.

§ Cells with “not applicable” in this column indicate that no MRD model was fit as part of the cited study.

4.4 Methods

4.4.1 Microcosm MRD Model Formulation

The microcosm system modeled herein is based upon experiments performed to quantify MRD Monod parameters for aquifer materials sampled at a site in Vermont.^{143,156} Experiments were performed in 160-mL capped glass serum bottle reactors prepared with site groundwater (approximately 100 mL) and 20 g of aquifer material and amended with TCE and sodium DL-lactate, which served as a fermentable substrate and was supplied at a concentration sufficient to

obviate the need to model electron donor limitations. The aqueous phase was bioaugmented with 0.033 mL of KB-1® inoculum (SiREM; Guelph, Ontario), which contains dechlorinators from the genera *Geobacter* (Geo) and *Dehalococcoides* (Dhc), fermenters, and other anaerobic bacteria.^{150,175,176} Additional experimental details can be found in Yang et al.¹⁴³

Geo can only convert PCE to TCE and TCE to cis-DCE.^{176,177} Although the Dhc strains in the KB-1® inoculum are collectively capable of converting PCE to ethene^{176,177}, it was assumed here that Dhc reductively dechlorinated only cis-DCE and VC, based upon microcosm experiments that demonstrated measurable Dhc population growth only during cis-DCE and VC degradation¹⁷⁰ and found that 80% of TCE degradation was attributable to Geo¹⁷⁷.

Under excess electron donor conditions, the MRD rate of CAH compound i can be represented by a Monod expression¹⁵⁰:

$$r_i = \frac{u_i^{\max} X_j^a C_i^a}{K_i^s + C_i^a} \quad (1)$$

where $u_i^{\max}$ is the maximum substrate utilization rate [mmol/(mg cell)/day], X_j^a is the biomass concentration [(mg cell)/L] of the microbe j that mediates the dechlorination, C_i^a is the aqueous concentration [mM] of CAH compound i , and K_i^s is the half-saturation constant [mM]. (Note that Equation 1 assumes no inhibition, consistent with the superior fitting performance of this model to the original microcosm data.¹⁴³ However, many forms of Equation 1 that include competitive inhibition have been posited in the literature¹⁵⁰ and could be used in the mass loss modeling technique described below.)

The overall reaction rate R_i^a for an aliphatic hydrocarbon is a combination of the individual MRD rates that change its aqueous concentration, i.e., $R_{\text{PCE}}^a = -r_{\text{PCE}}$, $R_{\text{TCE}}^a = r_{\text{PCE}} - r_{\text{TCE}}$, $R_{\text{DCE}}^a = r_{\text{TCE}} -$

r_{DCE} , $R_{\text{VC}}^a = r_{\text{DCE}} - r_{\text{VC}}$, and $R_{\text{ethene}}^a = r_{\text{VC}}$. Note that all the MRD models used and described in this paper assume ethene is not degraded, which has been demonstrated to occur in some anoxic conditions¹⁷⁸.

Under Monod kinetics, the population of a dechlorinating microbe j increases according to:

$$\frac{d}{dt}(X_j^a) = \sum_i (Y_i r_i) - k_j^b X_j^a \quad (2)$$

where the summation is over all CAH compounds that microbe j dechlorinates, Y_i is the yield coefficient [(mg cell)/(mmol CAH substrate)] for microbe j metabolizing CAH i , and k_j^b is an endogenous decay constant [1/day] for microbe j .

The mass balance equation for aliphatic hydrocarbon i in the microcosm is:

$$\frac{d}{dt}(V^a C_i^a) + \frac{d}{dt}(V^g C_i^g) + \frac{d}{dt}(q_i m_s) = V^a R_i^a - Q^a C_i^a - k_i^{dl} C_i^a \quad (3)$$

where V^k is the (time-varying) volume [L] of phase k (aqueous (a) or gaseous (g)), C_i^k is the concentration [mM] of compound i in phase k at time t , q_i is the soil sorption density [(mmol i)/(g soil)], and m_s is the mass of soil [g]. The last two terms in Equation 3 represent aqueous sampling and diffusive losses. Here the periodic removal of aqueous phase samples is approximated by a time-averaged constant extraction rate Q^a [L/day], and diffusive loss of compound i through the septum is assumed to have a first-order dependence¹⁴³ on C_i^a , with a diffusional mass loss rate coefficient k_i^{dl} [L/day], estimated using abiotic microcosm data (see Section 2.4).

Note that modeling the microcosm as a closed system implies that sampling and diffusive losses can be neglected (i.e., $Q^a = 0$; $k_i^{dl} = 0$).

For linear equilibrium partitioning between phases, rearranging Equation 3 (see Section 2.2) yields:

$$\frac{d}{dt}(C_i^a) = \frac{V^a R_i^a + \frac{C_i^a Q^a}{H_i R T} + k_i^{dl} C_i^a}{V^a + \frac{V^g}{H_i R T} + K_i^d m_s} \quad (4)$$

where H_i is the Henry's law constant [mmol/m³/Pa], R is the gas constant [J/K/mmol], T is temperature [K], K_i^d is the soil sorption linear partition coefficient [L/g] and V^a and V^g are computed as $V_0^a - Q^a t$ and $V_0^g + Q^a t$, respectively, where the subscript 0 indicates initial volumes. If methane or carbon dioxide forms and the microcosm becomes highly pressurized, H_i values may need to be adjusted with time (see Section 4.7).^{179,180}

A set of six ordinary differential equations (ODEs) can be solved for aliphatic hydrocarbon and biomass concentrations: Equation 4 for TCE, cis-DCE, VC, and ethene and Equation 2 for Geo and Dhc. These ODEs were integrated via an explicit Runge-Kutta algorithm¹⁸¹ of order 4(5), implemented in SciPy¹⁸².

4.4.2 MRD Model Parameter Estimation

The above microcosm model was used to estimate MRD kinetic parameters from laboratory-measured and computer-generated microcosm data. A differential evolution optimization algorithm¹⁸³, implemented in SciPy¹⁸², was applied to estimate selected model parameters by minimizing the sum of squared residuals (SSR):

$$SSR = \sum_{i=1}^4 \sum_{m=1}^n (C_{i,m}^{a,measured} - C_{i,m}^{a,modeled})^2 \quad (5)$$

where n is the total number of samples. Here the SSR is calculated with aqueous concentrations because the original microcosm¹⁴³ used an aqueous phase sampling scheme. Alternative optimization approaches based on a weighted SSR of GC and biomass concentration data^{143,170} were not considered herein. In the present work, fitting was confined to the Monod parameters u_{DCE}^{max} , u_{VC}^{max} , K_{DCE}^S , and K_{VC}^S , parameters that can be estimated from C_i^a measurements (Equation 5).¹⁵³ All biomass related parameters were adopted from the literature.

An initial model fit (see Section 4.8) to microcosm data¹⁴³ was used to obtain a set of “true” u_{DCE}^{max} , u_{VC}^{max} , K_{DCE}^S , and K_{VC}^S parameter values that could serve as estimation targets for numerical model fitting experiments described below. Mass loss model parameters were determined from abiotic microcosm controls, and other Monod parameters were taken from the literature (see Table 4.2). Using this approach, however, only the ratio of u_{VC}^{max} and K_{VC}^S could be fit uniquely, due to the small magnitude of C_{VC}^a in relation to K_{VC}^S (see Equation 1).^{150,184} (In practice, such best-fit parameter correlation issues can be ameliorated by modifying the experimental design to include higher initial CAH concentrations.¹⁵³) Given this constraint, K_{VC}^S was fixed at 0.0275 mM (half the peak measured C_{VC}^a and within the literature range^{146,164,170}) for model fitting of u_{DCE}^{max} , u_{VC}^{max} , and K_{DCE}^S . The resultant four parameters were taken as “true” values for subsequent simulations.

Table 4.2: Microcosm MRD Model Parameters

parameter	“true” values	note	optimization range†
maximum substrate utilization rates (mmol/(mg cell)/day)			
$u_{TCE}^{\max}$	5.58E-1	fixed (literature estimate ¹⁴³)	not applicable
$u_{DCE}^{\max}$	6.98E-1	calibration parameter	1.00E-3 to 1.06E0
$u_{VC}^{\max}$	2.74E-1	calibration parameter	6.00E-3 to 4.28E-1
half-saturation constants (mM)			
K_{TCE}^S	5.00E-3	fixed (averaged literature value ^{146,164,170,185})	not applicable
K_{DCE}^S	8.88E-2	calibration parameter	1.00E-3 to 1.96E-1
K_{VC}^S	2.75E-2	calibration parameter	1.00E-3 to 1.97E-1
microbial yield coefficients (mg cell/mmol substrate)			
Y_{TCE}	1.03E+1	fixed (averaged literature value ^{146,164,170,177})	not applicable
Y_{DCE}	1.06E-1	fixed (literature estimate ¹⁴³)	not applicable
Y_{VC}	9.60E-1	fixed (literature estimate ¹⁴³)	not applicable
endogenous decay constants (day ⁻¹)			
k_{Geo}^b	5.00E-2	fixed (common literature value ^{143,146,159,170,186})	not applicable
k_{Dhc}^b	6.60E-2	fixed (literature estimate ¹⁴³)	not applicable
effective diffusional mass loss rate coefficient (L/day) ‡			
k_{TCE}^{dl}	1.87E-4 / 0 (LML) / 8.77E-4 (HML)	fixed (abiotic experiment ¹³ /assumed)	not applicable
k_{DCE}^{dl}	2.18E-4 / 0 (LML) / 1.02E-3 (HML)	fixed (abiotic experiment ¹³ /assumed)	not applicable
k_{VC}^{dl}	2.72E-4 / 0 (LML) / 1.27E-3 (HML)	fixed (abiotic experiment ¹³ /assumed)	not applicable
k_{ethene}^{dl}	4.06E-4 / 0 (LML) / 1.90E-3 (HML)	fixed (abiotic experiment ¹³ /assumed)	not applicable
aqueous phase sampling rate (L/day)			
Q^a	3.00E-4 L/day	fixed (sampled every 3 days)	not applicable

† Optimization ranges are based on literature ranges for studies that used KB-1® inoculum^{146,164,170}. Upper bounds for $u_{DCE}^{\max}$ and $u_{VC}^{\max}$ were set to twice the literature maximum to better ensure that a global minimum of the objective function was found.

‡ LML = low mass loss and HML = high mass loss

4.4.3 Monte Carlo Model Fitting Experiment

A schematic of the Monte Carlo experiment procedure is presented in Figure 4.1. First, the system of six ODEs (Equations 2 and 4) was solved for two different mass loss scenarios (i.e., with differing k_i^{dl} parameters) (see Table 4.2). Here the low mass loss (LML) scenario (10% loss) assumed sampling occurred every 3 days without diffusional mass loss, and the high mass loss

(HML) scenario (40% loss) assumed the same sampling frequency, but included significant diffusional mass loss through the septum. In the HML scenario, the k_i^{dl} were scaled using Graham's law to achieve a total mass loss of 40% over 60 days. Random error was then added to the regularly spaced (3 days) solution data points, producing 663 synthetic GC measurement datasets for each mass loss scenario. The added error approximated GC analytical measurement error and was assumed to follow a normal distribution with a zero mean and constant coefficient of variation (COV) for each aliphatic hydrocarbon. The COVs for cis-DCE and VC (2.9% and 3.9%, respectively) were estimated by extracting data from published GC calibration curves¹⁸⁷ and fitting a linear regression to the sample standard deviations for each standard solution. The COV for ethene (3.9%) was assumed equal to that for VC.

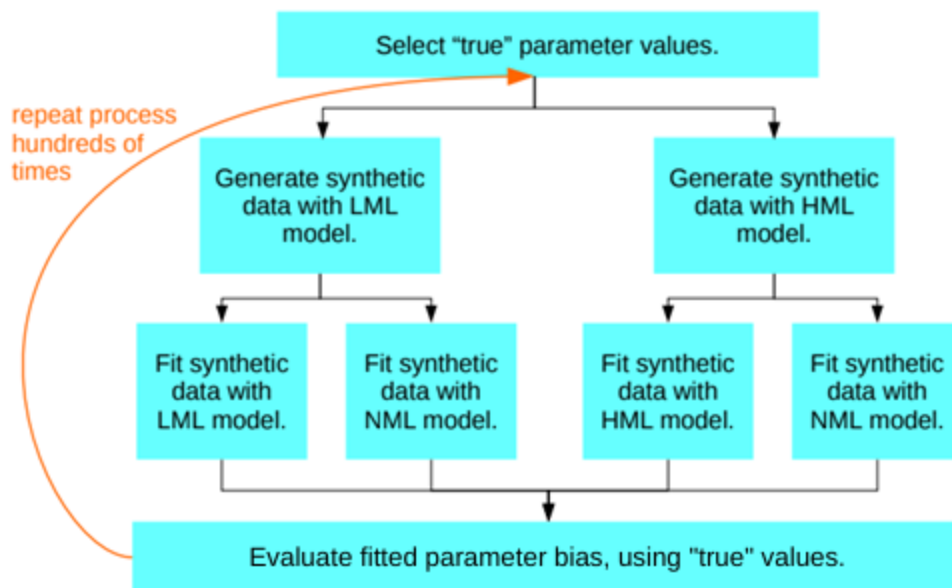


Figure 4.1: Overall Procedure for Monte Carlo Model Fitting Experiment

Best-fit values for each fitting parameter ($u_{DCE}^{\max}$, $u_{VC}^{\max}$, K_{DCE}^S , and K_{VC}^S) were then determined for every synthetic dataset (663×2) by independently fitting a model that accurately accounted for mass loss (i.e. a model with mass loss parameters Q^a and k_i^{dl} identical to the model used to

generate the synthetic data (LML or HML)) and a model that assumed no mass loss (NML). Each model fit began with a different randomly generated population of initial guesses and employed fixed optimization bounds, based upon literature ranges for KB-1® inoculum studies^{146,164,170} (Table 4.2). Model fitting results for each synthetic dataset were then compared to investigate fitted parameter bias when mass loss was neglected.

4.4.4 Model Fitting Experiments with Different “True” Parameter Values

To explore the sensitivity of the Monte Carlo fitting results to the “true” kinetic parameter values, additional fitting experiments were conducted for twenty alternative sets of “true” model parameters (see Table 4.4). These alternative parameters were selected from within the range of reported literature values for MRD microcosm experiments with the KB-1® inoculum^{146,164,170} and obtained by multiplying each original “true” parameter value by a perturbation factor. Perturbation factors for each fitting parameter were selected to return a wide variety of alternative parameter values distributed across literature ranges:

- $u_{\text{DCE}}^{\text{max}}$: 0.4, 0.5, 0.6, 0.7, and 1.0
- $u_{\text{VC}}^{\text{max}}$: 0.4, 0.5, 0.6, 0.7, and 1.0
- $K_{\text{DCE}}^{\text{S}}$: 0.5, 0.75, 1.0, 1.25, and 1.5
- K_{VC}^{S} : 0.3, 0.5, 1.0, 2.0, 3.0, and 4.0

Here perturbation factors for $u_{\text{DCE}}^{\text{max}}$ and $u_{\text{VC}}^{\text{max}}$ are ≤ 1.0 because the initial “true” values were at the top of the literature ranges. All 750 ($5 \times 5 \times 5 \times 6$) possible combinations of these perturbation factors were evaluated for use in the additional model fitting experiments. From these combinations, twenty parameter sets were selected to be representative of the range of possible parameter values observed in the literature, while ensuring “complete dechlorination” (i.e., 95% CAH reduction to

ethene) within 300 days. Both HML (40% mass loss) and LML (10% mass loss) datasets were generated (by integration of Equations 2 and 4) for each alternative parameter set and then used to estimate parameters in a process identical to one trial of the Monte Carlo experiment as depicted in Figure 4.1. See Table 4.4 for generated parameter sets.

4.5 Results and Discussion

4.5.1 Monte Carlo Model Fitting Experiment

Figure 4.2 presents example Monte Carlo-generated synthetic datasets and model fits for an LML/HML scenario pair, along with measured versus simulated total CAH. Inspection of the figure reveals that the LML/HML model fits (dashed lines) to the LML/HML datasets generally capture the magnitude, timing, and curvature of the aliphatic hydrocarbon data (Figures 4.2a and 4.2b). In contrast, fitting a model with no mass loss (NML) to the LML datasets (Figure 4.2a, solid lines), while capturing the timing and curvature of the data and its magnitude for cis-DCE and VC, produce a modeled ethene consistently higher than the measurements. This discrepancy, however, is likely not sufficient to suggest model structural issues – many modelers would characterize this as a “good” fit (especially if GC measurements ceased when >95% of VC was dechlorinated). NML model fits to the HML datasets (Figure 4.2b) exhibited similar behavior but with more pronounced discrepancies, consistently overpredicting concentrations of cis-DCE, VC, and ethene.

Each set of fitted model curves plotted in Figure 4.2 is associated with a unique set of best-fit values for $u_{\text{DCE}}^{\text{max}}$, $u_{\text{VC}}^{\text{max}}$, $K_{\text{DCE}}^{\text{S}}$, and K_{VC}^{S} . Figures 4.2c and 4.2d present the fitted parameter values associated with these curves. Here each axis corresponds to one of the four fitting parameters. Axis limits are consistent with parameter ranges used to constrain the fitting process. Recall that the same “true” values for $u_{\text{DCE}}^{\text{max}}$, $u_{\text{VC}}^{\text{max}}$, $K_{\text{DCE}}^{\text{S}}$, and K_{VC}^{S} (orange dots) were used to generate all synthetic datasets. Examination of the figure reveals the superior performance of the models that

appropriately account for mass loss in comparison to the NML model. The best-fit parameter values from the LML model fit to an LML scenario have an absolute error ranging from 1% to 30% in contrast to the corresponding best-fit values from the NML model that have absolute errors ranging from 8% to 51%. Similarly, for the HML case, the best-fit values from the HML model outperformed the NML model (absolute error ranges of 2% to 44% and 25% to 121%, respectively), with the NML best-fit K_{DCE}^S and K_{VC}^S pinned to the optimization bounds.

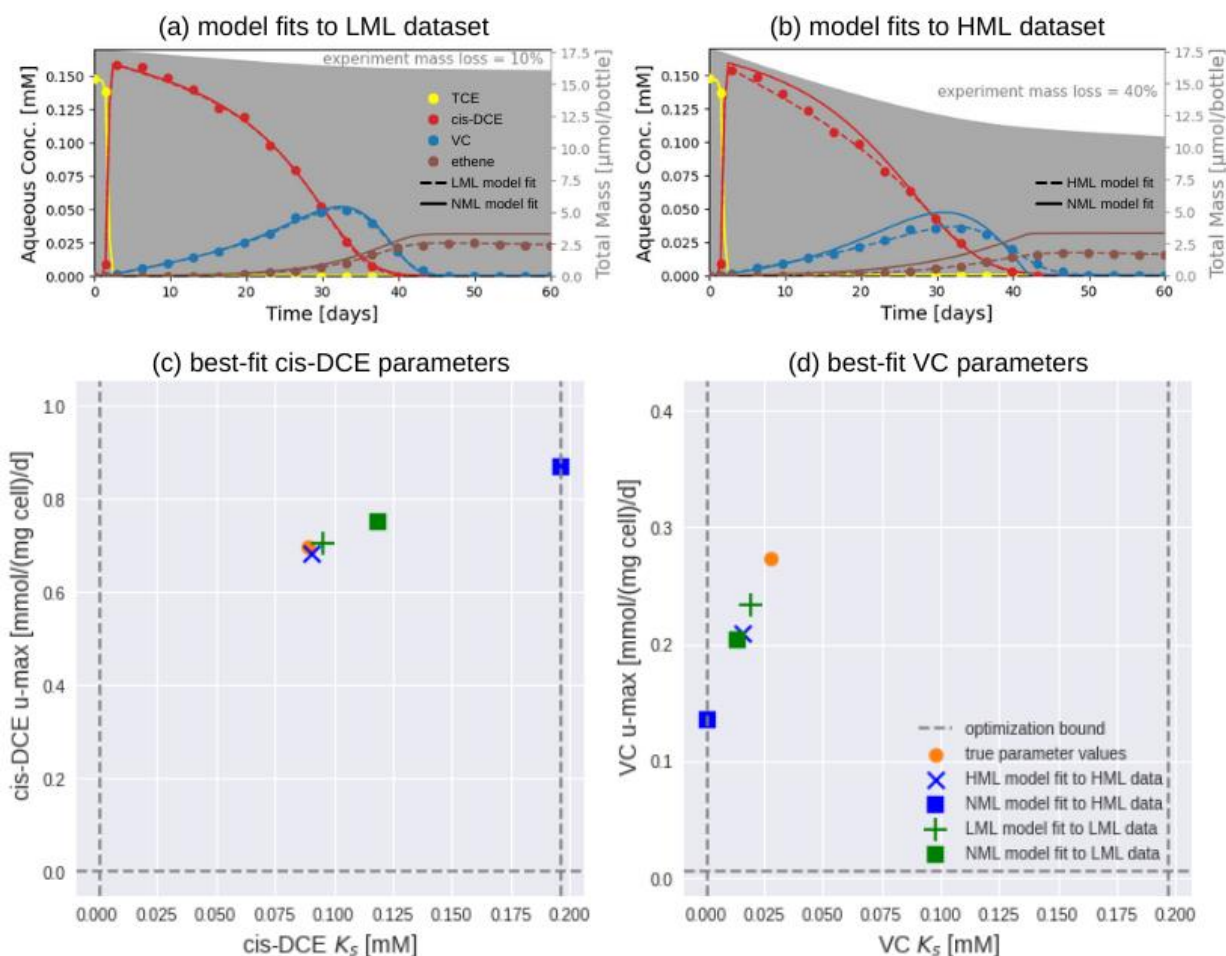


Figure 4.2: Fits for Single Trial of Monte Carlo Experiment. (a) “true” LML data (symbols) and NML (solid line) and LML model (dashed) fits; (b) “true” HML data (symbols) and NML (solid line) and HML model (dashed) fits; Best-fit model and “true” parameter values for cis-DCE (c) and VC (d). See Table 4.2 for optimization ranges. Curves and points plotted in Figures 4.2a and 4.2b indicate aqueous phase concentrations (left axis) and the height of the gray shaded area in those plots indicates total aliphatic hydrocarbons remaining in the bottle (right axis).

Figure 4.3 summarizes the results of independent model fits to all 663 LML/HML pairs of synthetic datasets. Here best-fit parameters are plotted as a “heatmap”, where the degree of darkness of each pixel represents the density of best-fit parameters in that region. The “true” parameter values used to generate the synthetic datasets are plotted as orange points and mean best-fit parameter values are plotted as brown crosshairs. The suite of Monte Carlo fitting results generally exhibits the same behavior as the illustrative example presented in Figure 4.2, with best-fit parameters for the models with mass loss (Figures 4.3a, 4.3c, 4.3e, and 4.3g) more closely centered about the “true” values than the closed-system models (Figures 4.3b, 4.3d, 4.3f, and 4.3h). The total error of any parameter estimator (in this context the entire model-fitting process that takes GC measurements as input and returns best-fit parameter values as output) is the sum of two distinct terms: variance and bias.¹⁸⁸ Variance is a measure of the spread of individual best-fit parameter estimates about the mean estimated value, and bias is the difference between that mean parameter estimate and the true parameter value. If a parameter estimator simultaneously determines best-fit values for multiple parameters, then fully characterizing the variance necessitates measuring the correlation between above-average estimates for one parameter and above- or below-average estimates for the other parameters.¹⁸⁹

For example, Figures 4.3a and 4.3c show that best-fit $u_{\text{DCE}}^{\text{max}}$ and $K_{\text{DCE}}^{\text{S}}$ for the models with mass loss tend to concentrate in an elongated ellipsoidal region, with a centroid that nearly coincides with the “true” $K_{\text{DCE}}^{\text{S}}$ and $u_{\text{DCE}}^{\text{max}}$ values. The diagonal orientation of this ellipsoidal region indicates that $K_{\text{DCE}}^{\text{S}}$ estimates are positively correlated with $u_{\text{DCE}}^{\text{max}}$ estimates. Similarly, mean HML and LML model best-fit values for the VC parameters (Figures 4.3e and 4.3g) also nearly coincide with (i.e. are within 6% of) the “true” K_{VC}^{S} and $u_{\text{VC}}^{\text{max}}$ values, but individual estimates exhibit a higher degree

of positive correlation than the cis-DCE parameters. Such correlation between fitting parameters is one of the primary obstacles that can confound accurate estimation of Monod kinetic coefficients.¹⁵³ Despite this strong correlation, the differential evolution algorithm¹⁸³, used herein for model fitting, robustly avoids local minima by evaluating many sets of potential fitting parameter values in parallel and was able to determine a unique best-fit parameter set, associated with the objective function global minimum (Equation 5), for each Monte Carlo trial. Repeated optimizations for a selection of model fitting experiments, commencing with different suites of randomly generated initial parameter guesses, returned consistent best-fit parameters.

In contrast, use of a closed-system model to fit the data resulted in a substantial increase in parameter variance and a reduction in estimated parameter accuracy. For the NML model fit to the LML scenario datasets, the variance of cis-DCE parameter estimates clearly increased (compare ellipsoidal regions in Figures 4.3d and 4.3c). The greater effect, however, was an increase in bias of the mean cis-DCE and VC parameter estimates (departure from the “true” values). Fitting the NML model to the HML scenario datasets resulted in even greater parameter estimation bias. Here the bias was so great as to prohibit evaluation of the parameter estimation variance; in every model fit, K_{DCE}^S was pinned to its upper optimization bound with $u_{DCE}^{max} \cong 0.862$ mmol/(mg cell)/d, and K_{VC}^S was pinned to its lower bound with $u_{VC}^{max} \cong 0.135$ mmol/(mg cell)/d. Regardless of mass loss extent, best-fit u_{DCE}^{max} and K_{DCE}^S parameters for the NML models tend to have a positive bias, while the corresponding best-fit u_{VC}^{max} and K_{VC}^S parameters tend to have a negative bias. The magnitude of the bias appears to be roughly proportional to the magnitude of the (neglected) mass loss.

As the neglected mass loss percentage increases, the NML model system has more mass than is present in the true system, which results in the aqueous concentration of one or more aliphatic hydrocarbons being overpredicted. This potential for overprediction is greatest for VC and ethene

because they are the primary compounds present at later times, when experimental mass loss is greatest. Thus, the fitting process will drive production rates lower for these lesser chlorinated compounds to reduce the objective function (Equation 5). For example, if a constant average value for X_{Dhc}^a is assumed, transformation rates r_i (as a function of concentration C_i^a) can be calculated from Equation 1 using “true” parameter values and mean NML best-fit parameters. For the LML scenario these calculations reveal that the NML model results in 2-14% lower r_{DCE} values over the entire cis-DCE concentration range and up to 4% lower r_{VC} values (for $C_{VC}^a \geq 0.024$ mM). A similar calculation for the HML scenarios yields even greater artificial depression of the cis-DCE and VC transformation rates: up to 41% and 25% for r_{DCE} and r_{VC} , respectively.

To perform a quantitative comparison of fitted parameter values for each individual dataset, added absolute error (AAE), was defined as:

$$AAE(\theta_p) = \frac{|\theta_p^{NML} - \theta_p^{true}| - |\theta_p^{ML} - \theta_p^{true}|}{\theta_p^{true}} \times 100\% \quad (6)$$

where θ_p corresponds to one of the four fitting parameters ($\theta_p = u_{DCE}^{max}, u_{VC}^{max}, K_{DCE}^S$, or K_{VC}^S), θ_p^{true} is the “true” value of that parameter, θ_p^{NML} is the best-fit value of parameter θ_p for the NML model, and θ_p^{ML} is the corresponding best-fit value for the model with mass loss. A positive AAE indicates that the model with mass loss outperformed the NML model, with best-fit parameters closer to the “true” values.

Using this metric, the LML model outperformed the NML model for all four fitting parameters: out of 663 LML datasets, 66%, 79%, 67%, and 67% had positive AAE values for u_{DCE}^{max} , u_{VC}^{max} , K_{DCE}^S , and K_{VC}^S , respectively. Figures 4.4a and 4.4b present heatmaps of AAE for LML scenario datasets. Here the darkness of each pixel represents the number of datasets that had AAE values in that

particular range. The blue point on each heatmap designates the mean AAE value for all 663 LML datasets. As with the fitted parameter values themselves (see Figure 4.3), positive AAE values tend to cluster in linear regions. Similarly, the HML model greatly outperformed the NML model for all four fitting parameters: 99.8%, 97.9%, 100.0%, and 97.7% of datasets had positive AAE values for $u_{\text{DCE}}^{\text{max}}$, $u_{\text{VC}}^{\text{max}}$, K_{DCE}^s , and K_{VC}^s , respectively (Figures 4.4c and 4.4d). Consistent with the LML results, positive AAE values were clustered in linear regions, but average AAE values were significantly larger.

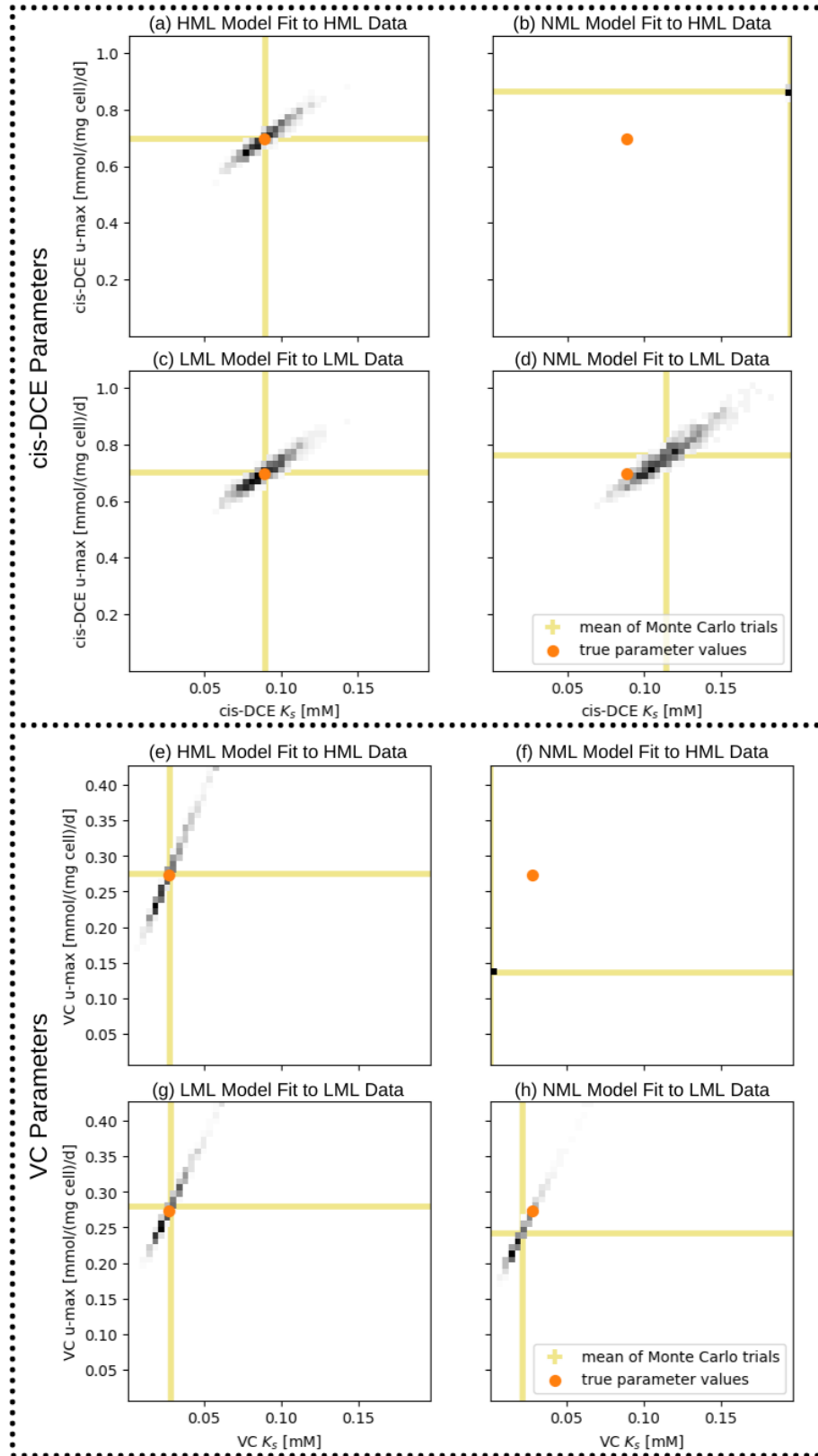


Figure 4.3: Heatmap of Best-Fit Parameters from Monte Carlo Experiment

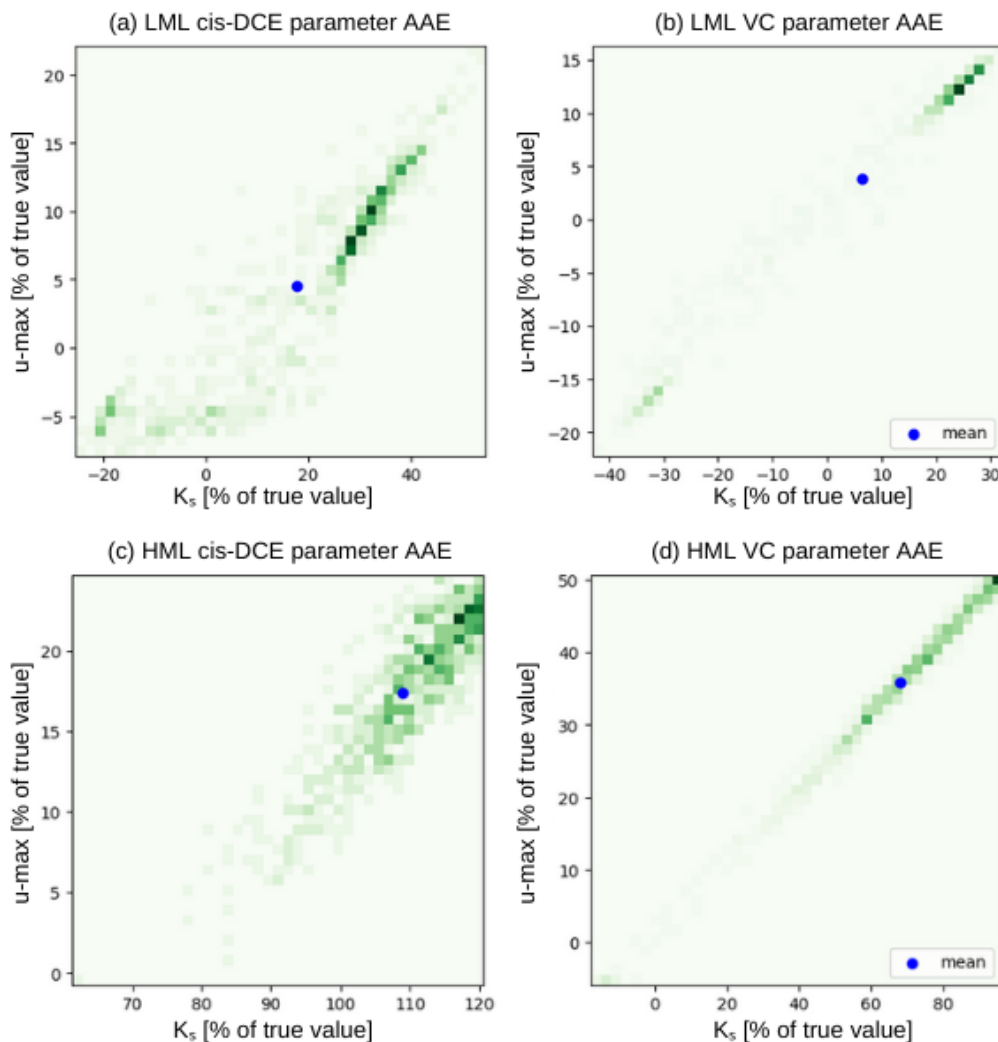


Figure 4.4: Heatmaps of Added Absolute Error for Models Fit to LML and HML Datasets in the Monte Carlo Experiment

4.5.2 Model Fitting Experiments with Alternative "True" Parameter Values

The same analysis using the 20 alternative parameter sets demonstrated that the above results were not an artifact of the initial “true” parameters selected. Results from the additional 20 model fitting experiments indicated that NML model parameter estimation error is a concern even when experimental mass loss is only 10%. As illustrated in Figures 4.5a and 4.5b, the majority of AAE values for all four fitting parameters were positive. Mean AAE values were 3%, 3%, 9%, and 14%

for $u_{\text{DCE}}^{\text{max}}$, $u_{\text{VC}}^{\text{max}}$, K_{DCE}^s , and K_{VC}^s , respectively. A one-tailed significance test¹⁸⁹ using the t-distribution with 19 degrees of freedom was used to test the null hypothesis that the mean AAE for a parameter is zero (i.e. a null hypothesis that the LML model does no better on average than the NML model); this null hypothesis can be rejected with confidence levels of 99%, 88%, 98%, and 99% for $u_{\text{DCE}}^{\text{max}}$, $u_{\text{VC}}^{\text{max}}$, K_{DCE}^s , and K_{VC}^s , respectively.

As before, fitting an NML model to an HML dataset resulted in pinned fitting parameters in all 20 of the additional model fitting experiments (see Table 4.5). This indicates that strong parameter estimation bias should be expected when a closed-system model is fit to microcosm data with a high degree of experimental mass loss. Although such pinning behavior has not been widely reported in the literature, most papers reviewed for this study include limited descriptions of the model fitting process, making it challenging to determine if and how optimization bounds were developed and applied. Figures 4.5c and 4.5d present scatter plots of AAE for the HML datasets. Here all but one of the fitting experiments had positive AAE values for all four fitting parameters, confirming that the model with mass loss consistently outperformed the closed-system model. Mean AAE values were 40%, 35%, 184%, and 116% for $u_{\text{DCE}}^{\text{max}}$, $u_{\text{VC}}^{\text{max}}$, K_{DCE}^s , and K_{VC}^s , respectively. As observed for the first Monte Carlo experiment, estimated average r_{DCE} and r_{VC} values were both lower than true values for 16 out of 20 of the HML and LML datasets. No such pattern was observed for the models with mass loss.

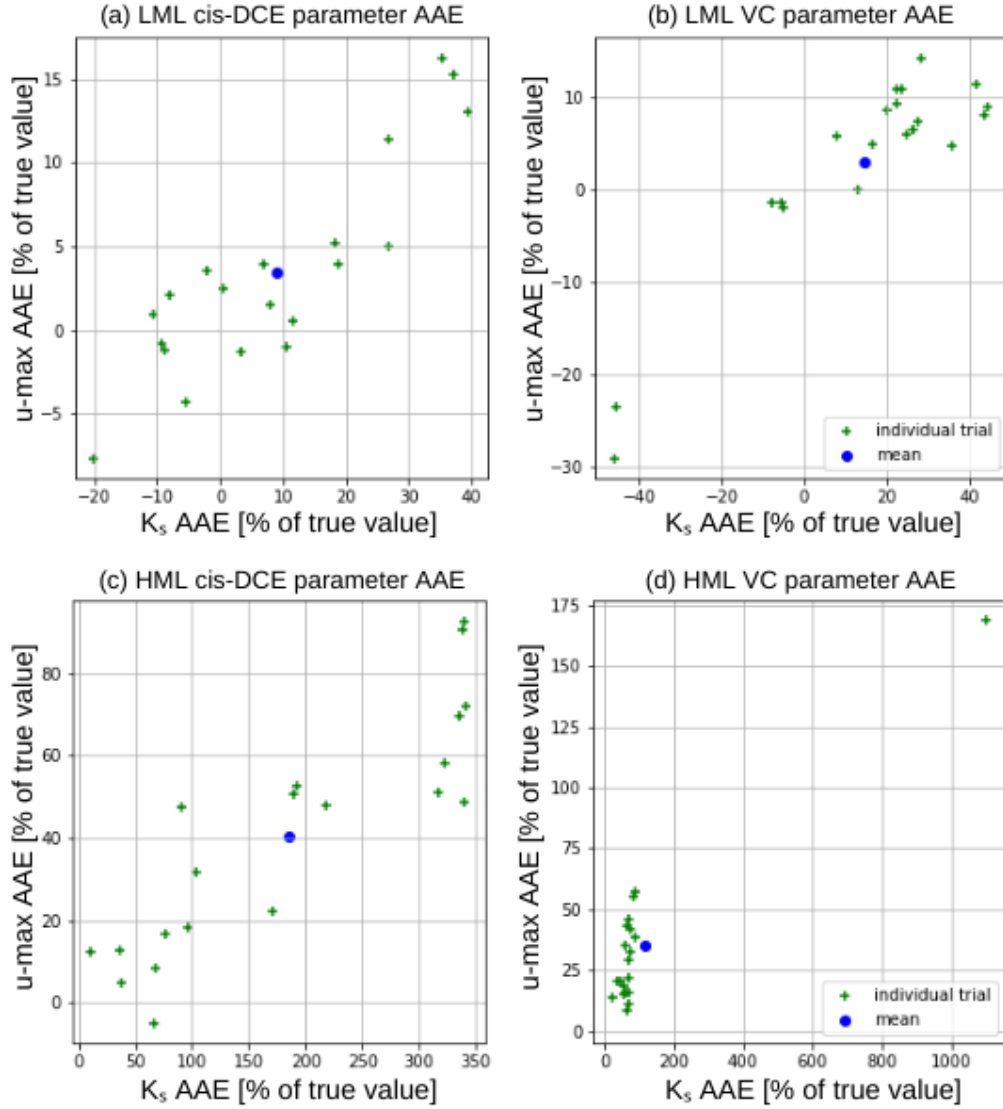


Figure 4.5: Plots of Added Absolute Error for Model Fitting Experiments to LML and HML Datasets with Different “True” Parameter Values

It has been demonstrated¹⁵³ for a similar inverse problem that individual estimation variances of K_i^S and $u_i^{\max}$ (i.e., the width of the ellipsoidal cluster of probable best-fit parameter estimates (e.g., see Figure 4.3a)) are directly proportional to substrate measurement error and are a complex nonlinear function of C_i^a/K_i^S and $X_j^a/(Y_i K_i^S)$. The same study showed that correlation between K_i^S

and $u_i^{\max}$ (i.e. the ratio of the major and minor axes of that ellipsoid) depended only on C_i^a/K_i^s and $X_j^a/(Y_iK_i^s)$. As described in the Methods section, all model-fitting experiments in this paper used synthetic data generated with the same levels of assumed GC measurement error. Assuming that the degree of fitting parameter correlation is approximately the same for models with and without mass loss considerations (as suggested by Monte Carlo experiments with the LML datasets), increasing the GC measurement error would be expected to lead to a proportional increase in the size of the regions of probable best-fit parameter estimates. However, it would not be expected to change the shape of those regions nor the mean best-fit parameter estimates. For scenarios where substrate measurement error is large and experimental mass loss is small, parameter interval estimates for models with and without mass loss may prove difficult to distinguish. The precise substrate measurement error and experimental mass loss thresholds at which this would occur, however, cannot be determined from the foregoing analysis and may differ for alternative model-fitting scenarios. Thus, given this uncertainty and the relative ease of accounting for experimental mass loss, the practical advantage of using a model with mass loss for parameter fitting is clear.

The MRD model employed in this paper assumes no competitive inhibition. It is unclear how parameter estimation biases caused by ignoring mass loss would manifest if the model formulation included inhibition, and investigating such biases could be the subject of future work.

Overall, results presented herein emphasize the critical importance of accounting for mass loss in microcosm model formulations. Microcosm studies like those detailed in the literature review have played a pivotal role in developing ISB technology into a viable remediation alternative, but caution is advised when interpreting the large body of published modeling work that has employed a closed-system modeling assumption, especially if the associated experimental mass losses exceeded 10%. Results presented herein suggest that kinetic parameter estimates based

upon closed-system models will be biased and tend to underpredict ISB efficacy or culture performance. However, for more complicated inverse problems (i.e. those with more fitting parameters or those that employ biomass concentration data in fitting) it is difficult to predict *a priori* how a closed-system assumption would degrade parameter estimation accuracy.¹⁵⁵

4.6 Extended Literature Review and Analysis

Experimental measurements and model predictions plotted in the cited papers (Table 4.1, column 1) were extracted with WebPlotDigitizer¹⁵⁷ and used (1) to estimate experimental mass losses, if not quantified by the study authors (Table 4.1, column 4), and (2) to evaluate whether a paper's model fitting method accounted for these mass losses (Table 4.1, column 6). A subset of the studies did not have sufficient information to estimate experimental mass loss. If a study within that subset attempted to model the MRD process it is included in Table 4.1; other studies^{177,190–192} were excluded from the table. Two of the studies in Table 4.1 have an experimental mass loss of “ ≈ 0 ” (column 4). In one, presented measurements appear to have been corrected for experimental mass loss (Yu et al.¹³⁹), and in the other, presented data suggest an increase of mass over time (He et al.¹⁶⁵).

All reviewed studies tracked the MRD process over time by monitoring the aqueous concentration of each aliphatic hydrocarbon. Studies that attempted to estimate the total amount of each compound present in the microcosm (e.g. as a plot of moles/bottle versus time^{146,164,168}) did so by assuming equilibrium partitioning between the aqueous phase, gaseous headspace, and sorbed soil phase (if present, see column 3 of Table 4.1). All estimates of experimental mass loss in column 4 of Table 4.1 were made using this equilibrium partitioning assumption.

Measures that may reduce experimental mass loss do not appear to have been widely applied or highly effective. The only studies in this literature review that used Mininert valves to limit mass loss did not fit kinetic MRD models.^{173,176,177} Microcosms with Teflon-lined septa still exhibited mass losses greater than 25%.^{169,172} Studies that employed lower-volume headspace sampling regimes (see Table 4.3) typically lost 20% or more mass.^{159,166}

Other information, such as electron donor and experiment duration, are summarized for many of these studies in Chambon et al.¹⁵⁰

Table 4.3: Summary of Microcosm Sampling Methodologies

Reference Cited	Phase Sampled	Sample Volume [μ L]	Microcosm Volume [mL]
Smith ^{151,158}	gaseous	200-400	2000
Cupples ¹⁵⁹	gaseous	250	120
Cupples ¹³⁷	gaseous	250	120
Cupples ¹⁶⁰	gaseous	250	120
Yu ¹³⁹	gaseous	100-200	156
He ¹⁶⁵	gaseous	100-200	160
Yang, Y. ¹⁶⁶	gaseous	250	117
Yu ¹⁶⁷	gaseous	100-200	150
Amos ¹³⁸	aqueous	1000	160
Friis ¹⁴⁶	aqueous	1000	120
Friis ¹⁶⁸	aqueous	1000	500
Huang ¹⁶¹	gaseous	500	160
Schaefer ¹⁶²	gaseous	unknown	60-7000
Cichocka ¹⁶⁹	gaseous	500	120
Haest ¹⁷⁰	aqueous	1000	120
Sabalowsky ¹⁷¹	gaseous	100	156
Malaguerra ¹⁷²	aqueous	1000	320
Harkness ¹⁷³	aqueous	10-1000	250
Bælum ¹⁶⁴	aqueous	1000	320
Schneidewind ¹⁷⁴	gaseous	unknown	160
Yang, L. ¹⁴³	aqueous	1000	160

4.7 Microcosm Pressurization

In experimental microcosms where gas formation may occur the Henry's law coefficient H_2 at system pressure P_2 can be calculated with H_1 and P_1 via the expression

$$\frac{1}{H_2} = \frac{1}{H_1} \exp \left[\int_{P_1}^{P_2} \frac{\bar{V}^\infty}{RT} dP \right] \quad (7)$$

where $\bar{V}^\infty$ is the partial molar volume at infinite dilution, R is the gas constant, and T is temperature.¹⁸⁰ If it is assumed that $\bar{V}^\infty$ is invariant with system pressure¹⁷⁹ and can be approximated with the pure phase molar volume¹⁸⁰, then simplifying yields:

$$\frac{1}{H_2} \approx \frac{1}{H_1} \exp \left[\frac{V^m(P_2 - P_1)}{RT} \right] \quad (8)$$

where V^m is the pure phase molar volume calculated with the molar weight and tabulated density data¹⁹³. This correction factor is quite small, however, for most aliphatic hydrocarbons under typical experimental conditions.

For example, if the microcosm system described in this paper had an initial lactate concentration of 5 mM and each mole of lactate yielded two moles of carbon dioxide and one mole of methane¹⁹⁴ application of the ideal gas equation would suggest a pressure increase of less than 0.6 bar. Approximate Henry's law coefficient correction factors (i.e. the exponential in Equation 16) for such a pressure increase are 1.0025, 1.0022, 1.0019, 1.0017, 1.7979 for PCE, TCE, cis-DCE, VC, and ethene, respectively. These calculations suggest that such a correction would only be needed for H_{ethene} if the microcosm were extensively pressurized (which was not observed during any of the laboratory experiments that the synthetic data in this paper are based on).

It is worth noting, however, that if such a correction were neglected, then even a model with the correct mass loss considerations could still possibly overpredict C_{ethene}^a and underpredict C_{ethene}^g . Depending on the sampling methodology used (see Table 4.3), failure to correct H_{ethene} could increase parameter estimation error. Incorporating Equation 8 into the kinetic model presented here (Equation 4), however, provides a method for more accurately modeling kinetic systems even in the presence of a pressurized microcosm.

4.8 Initial Model Fit

Table 4.2 provides best-fit model parameter values from the initial model fit, which had a Sum of Squared Residuals (SSR) of $6.54\text{E-}3 \text{ mM}^2$ (see Equation 5). Figure 4.6 plots the corresponding modeled and measured aqueous concentrations for this initial scenario. The shapes of the cis-DCE and ethene measurements were well captured by the fitted model (SSR of $8.55\text{E-}4$ and $1.08\text{E-}4 \text{ mM}^2$, respectively, if the anomalous cis-DCE measurement on day 5 is excluded). Fitted model agreement with the VC measurements was not as good (SSR of $6.82\text{E-}4 \text{ mM}^2$), but the general timing of the rise and fall of the VC concentration, as well as its peak magnitude were generally consistent with the experimental observations. Figure 4.6 confirms that the fitted model parameters reproduce a realistic TCE to ethene degradation scenario in an experimental microcosm.

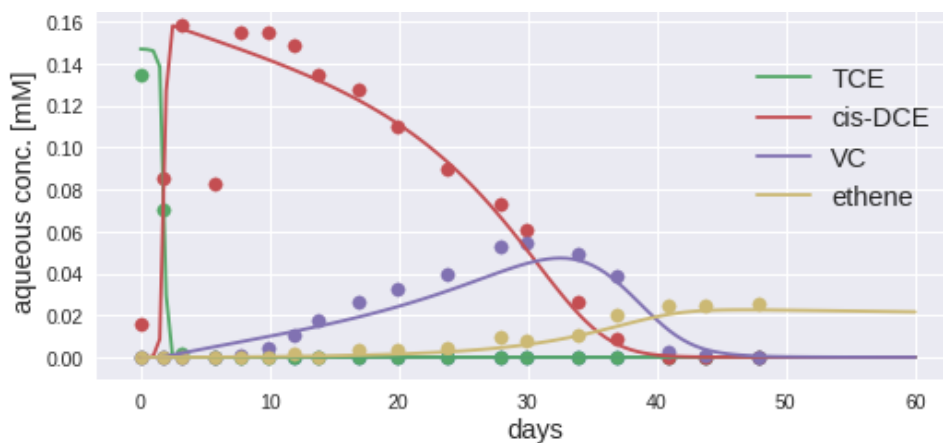


Figure 4.6: Initial Model Fit to Microcosm 2 Data from Yang et al.¹⁴³

Table 4.4: Alternative Parameter Values for Additional Model Fitting Experiments (All other parameters match those presented in Table 4.2.)

trial	maximum substrate utilization rate [mmol/(mg cell)/d]		half saturation coefficients [mM]		aqueous phase sampling rate [L/day]		HML effective diffusional mass loss rate coefficient [L/day]			
	$u_{DCE}^{\max}$	$u_{VC}^{\max}$	K_{DCE}^S	K_{VC}^S	LML Q^a	HML Q^a	k_{TCE}^{dl}	k_{DCE}^{dl}	k_{VC}^{dl}	k_{ethene}^{dl}
1	2.79E-1 (×0.4)	1.37E-1 (×0.5)	4.44E-2 (×0.50)	8.25E-3 (×0.3)	-1.46E-4	-1.33E-4	-4.54E-4	-5.28E-4	-6.58E-4	-9.82E-4
2	2.79E-1 (×0.4)	2.74E-1 (×1.0)	4.44E-2 (×0.50)	8.25E-3 (×0.3)	-2.77E-4	-2.65E-4	-8.38E-4	-9.76E-4	-1.22E-3	-1.81E-3
3	3.49E-1 (×0.5)	1.37E-1 (×0.5)	4.44E-2 (×0.50)	8.25E-3 (×0.3)	-1.75E-4	-1.71E-4	-5.64E-4	-6.57E-4	-8.18E-4	-1.22E-3
4	3.49E-1 (×0.5)	2.74E-1 (×1.0)	4.44E-2 (×0.50)	8.25E-3 (×0.3)	-3.40E-4	-3.27E-4	-1.02E-3	-1.19E-3	-1.48E-3	-2.20E-3
5	6.98E-1 (×1.0)	1.10E-1 (×0.4)	4.44E-2 (×0.50)	8.25E-3 (×0.3)	-1.84E-4	-1.88E-4	-6.77E-4	-7.88E-4	-9.81E-4	-1.46E-3
6	6.98E-1 (×1.0)	2.74E-1 (×1.0)	4.44E-2 (×0.50)	8.25E-3 (×0.3)	-4.86E-4	-5.00E-4	-1.59E-3	-1.85E-3	-2.30E-3	-3.43E-3
7	2.79E-1 (×0.4)	1.64E-1 (×0.6)	6.66E-2 (×0.75)	8.25E-3 (×0.3)	-1.62E-4	-1.37E-4	-4.76E-4	-5.54E-4	-6.90E-4	-1.03E-3
8	3.49E-1 (×0.5)	1.37E-1 (×0.5)	6.66E-2 (×0.75)	8.25E-3 (×0.3)	-1.59E-4	-1.45E-4	-4.95E-4	-5.76E-4	-7.17E-4	-1.07E-3
9	6.98E-1 (×1.0)	1.10E-1 (×0.4)	8.88E-2 (×1.00)	8.25E-3 (×0.3)	-1.64E-4	-1.64E-4	-5.84E-4	-6.80E-4	-8.47E-4	-1.26E-3
10	4.89E-1 (×0.7)	1.10E-1 (×0.4)	1.11E-1 (×1.25)	8.25E-3 (×0.3)	-1.24E-4	-1.03E-4	-3.93E-4	-4.58E-4	-5.70E-4	-8.51E-4
11	6.98E-1 (×1.0)	1.10E-1 (×0.4)	1.11E-1 (×1.25)	8.25E-3 (×0.3)	-1.57E-4	-1.51E-4	-5.43E-4	-6.32E-4	-7.87E-4	-1.17E-3
12	3.49E-1 (×0.5)	2.74E-1 (×1.0)	1.33E-1 (×1.50)	8.25E-3 (×0.3)	-2.07E-4	-1.62E-4	-6.03E-4	-7.03E-4	-8.75E-4	-1.31E-3
13	2.79E-1 (×0.4)	2.74E-1 (×1.0)	6.66E-2 (×0.75)	1.38E-2 (×0.5)	-2.00E-4	-1.71E-4	-5.79E-4	-6.74E-4	-8.39E-4	-1.25E-3
14	2.79E-1 (×0.4)	2.74E-1 (×1.0)	8.88E-2 (×1.00)	1.38E-2 (×0.5)	-1.71E-4	-1.25E-4	-4.73E-4	-5.51E-4	-6.86E-4	-1.02E-3
15	6.98E-1 (×1.0)	2.74E-1 (×1.0)	1.33E-1 (×1.50)	1.38E-2 (×0.5)	-3.40E-4	-3.21E-4	-1.02E-3	-1.18E-3	-1.47E-3	-2.20E-3
16	2.79E-1 (×0.4)	2.74E-1 (×1.0)	4.44E-2 (×0.50)	2.75E-2 (×1.0)	-1.62E-4	-1.27E-4	-4.52E-4	-5.27E-4	-6.56E-4	-9.79E-4
17	6.98E-1 (×1.0)	1.92E-1 (×0.7)	6.66E-2 (×0.75)	2.75E-2 (×1.0)	-2.20E-4	-2.00E-4	-7.11E-4	-8.27E-4	-1.03E-3	-1.54E-3
18	4.89E-1 (×0.7)	2.74E-1 (×1.0)	4.44E-2 (×0.50)	5.50E-2 (×2.0)	-1.82E-4	-1.38E-4	-5.30E-4	-6.17E-4	-7.68E-4	-1.15E-3
19	6.98E-1 (×1.0)	2.74E-1 (×1.0)	4.44E-2 (×0.50)	5.50E-2 (×2.0)	-2.37E-4	-2.05E-4	-7.48E-4	-8.71E-4	-1.09E-3	-1.62E-3
20	6.98E-1 (×1.0)	2.74E-1 (×1.0)	1.11E-1 (×1.25)	5.50E-2 (×2.0)	-1.84E-4	-1.29E-4	-5.14E-4	-5.98E-4	-7.45E-4	-1.11E-3

Note: Values given in parentheses indicate a perturbation factor applied to the “true” maximum substrate utilization rate or half saturation coefficient from the Monte Carlo Experiment (see Table 4.2).

Table 4.5: Parameter Estimation Errors for Additional Model Fitting Experiments

trial	HML model fit to HML data				NML model fit to HML data				LML model fit to LML data				NML model fit to LML data			
	$u_{DCE}^{\max}$	$u_{VC}^{\max}$	K_{DCE}^S	K_{VC}^S	$u_{DCE}^{\max}$	$u_{VC}^{\max}$	K_{DCE}^S	K_{VC}^S	$u_{DCE}^{\max}$	$u_{VC}^{\max}$	K_{DCE}^S	K_{VC}^S	$u_{DCE}^{\max}$	$u_{VC}^{\max}$	K_{DCE}^S	K_{VC}^S
1	1%	12%	0%	32%	73%	-30%	341%	-88%	-4%	2%	-14%	3%	-6%	-8%	-6%	-28%
2	2%	25%	1%	52%	95%	-45%	341%	-88%	6%	34%	11%	77%	10%	10%	30%	32%
3	-5%	-10%	-6%	-31%	74%	-25%	341%	-88%	-3%	-6%	-5%	-20%	-5%	-13%	5%	-48%
4	-1%	1%	-3%	0%	91%	-39%	341%	-88%	-3%	1%	-11%	3%	-2%	-12%	2%	-26%
5	-5%	0%	-15%	6%	52%	169%	107%	1109%	-3%	4%	-15%	25%	7%	8%	13%	60%
6	-4%	-9%	-6%	-29%	52%	-25%	223%	-88%	-1%	-2%	-4%	-4%	-3%	-13%	12%	-46%
7	-3%	-7%	-3%	-19%	56%	-36%	194%	-88%	-3%	-1%	-4%	-6%	-2%	-10%	8%	-28%
8	-1%	6%	-4%	19%	51%	-28%	194%	-88%	1%	7%	0%	22%	0%	-5%	11%	-17%
9	-14%	-6%	-24%	-25%	32%	-14%	121%	-88%	-12%	-2%	-25%	-11%	-13%	-10%	-14%	-55%
10	-7%	-5%	-9%	-19%	16%	-20%	77%	-88%	-8%	-5%	-10%	-23%	-7%	-11%	1%	-49%
11	-2%	-4%	0%	-18%	19%	-15%	77%	-88%	0%	-3%	4%	-16%	-1%	-12%	16%	-60%
12	5%	11%	9%	18%	-18%	56%	20%	-88%	-2%	-17%	-1%	-32%	18%	-15%	37%	-24%
13	-10%	-42%	-13%	-71%	-23%	56%	49%	-93%	-8%	-31%	-11%	-56%	-4%	-37%	6%	-64%
14	-10%	16%	-17%	17%	41%	-58%	121%	-93%	-2%	56%	-3%	85%	13%	56%	30%	98%
15	4%	-9%	9%	-18%	9%	-41%	47%	-93%	2%	-10%	8%	-24%	8%	-21%	26%	-47%
16	2%	8%	5%	13%	61%	-64%	327%	-96%	8%	38%	18%	68%	13%	9%	45%	22%
17	-14%	-22%	-23%	-48%	36%	-42%	194%	-96%	-16%	8%	-43%	17%	-9%	13%	-23%	34%
18	0%	7%	-2%	11%	49%	-64%	341%	-98%	-3%	8%	-13%	15%	7%	17%	20%	35%
19	1%	-24%	9%	-40%	52%	-58%	326%	-98%	8%	-5%	28%	-10%	21%	19%	67%	38%
20	-7%	-20%	-10%	-31%	2%	-63%	77%	-98%	-1%	-13%	0%	-21%	16%	-11%	38%	-16%

Notes:

- All errors are relative to and normalized by the alternative “true” parameter values given in Table 4.4.
- Values highlighted in yellow were pinned to the respective optimization boundary (see Table 4.2).

4.9 Acknowledgements

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5 On the Reliable Estimation of Sequential Monod Kinetic Parameters

The content of this chapter is derived from a paper by Jack L. Elsey, Eric L. Miller, John A. Christ, and Linda M. Abriola. This paper has been submitted to Environmental Science & Technology for review. The author conducted all modeling and a majority of the analysis for this paper.

5.1 Abstract

Dozens of studies have attempted to estimate the Monod kinetic parameters of microbial reductive dechlorination (MRD), but published values in the literature vary by 2-6 orders of magnitude. This lack of consensus in the literature could be attributed in part to nonoptimal experimental designs and limitations of parameter estimation techniques. Hamiltonian Monte Carlo was used herein to generate realistic parameters to produce more than 1 million sets of simulated microcosm data, which were then employed in model fitting experiments. Various experimental design and parameter estimation methods were tested to develop a methodology for reliably determining Monod kinetic parameters. Conventional triplicate microcosms resulted in parameter estimates marred by extremely high collinearity, resulting in poor estimation accuracy and precision. A novel experimental design, requiring the same number of analyses as conventional experiments but comprised of microcosms with varying initial chlorinated ethene concentrations, resulted in order-of-magnitude decreases in parameter uncertainty. Classical regression analysis techniques commonly used to calculate marginal confidence intervals contained true parameter values much less frequently than their nominal confidence levels. A Metropolis algorithm was shown to return more reliable parameter interval estimates.

5.2 Introduction

Chlorinated solvents tetrachloroethene (PCE) and trichloroethene (TCE) are present at more than half of hazardous waste sites proposed for inclusion on the EPA National Priorities List.^{4,5}

Microbial reductive dechlorination (MRD), the process by which anaerobic bacteria sequentially degrade PCE to TCE, *cis*-1,2-dichloroethylene (*cis*-DCE), vinyl chloride (VC), and finally harmless ethene, is an established means for remediating these sites.¹¹ MRD performance is typically modeled with Monod kinetics.¹⁹⁵ Difficulties in obtaining precise interval estimates for Monod kinetic parameters were first noted almost half a century ago.¹⁹⁶ Later studies^{153,184,197–199} demonstrated strong collinearity among fitting parameters of Monod kinetic models. Strong collinearity increases the variance of parameter estimates, widening marginal confidence intervals and increasing best-fit point estimation error.¹⁵³ In addition to this collinearity, there has also been a lack of consensus¹⁹⁵ in the MRD literature over typical ranges for Monod kinetic parameters, with parameter estimates spanning 2-6 orders of magnitude (see Table 5.1). Compounding the problem, there is an absence of MRD literature that effectively investigates strategies for increasing estimation reliability.

Several papers^{153,184,196,199–202} have conducted numerical model fitting experiments with synthetic datasets to evaluate the precision of estimated parameters. However, none of these based their experiments on more than a few (1-5) sets of Monod parameters from the large potential range of values, nor have they evaluated whether their numerical model fitting experiment trials are statistically representative. Most prior work had fewer than 60 model fitting trials, with only one paper exceeding 1000.²⁰⁰ However, convergence of the Monte Carlo experiment (MCE) in the latter paper was not assessed. Among papers that generated synthetic datasets with known “true” parameter values, few papers summarized parameter estimation error. Many modeling studies have used regression analysis (RA) to calculate parameter confidence intervals²⁰³, but other than remarking on good agreement with statistical assumptions^{152,204}, coverage (a known potential weakness^{155,205,206}) has never been assessed.

Although some studies have employed principles of optimal design^{207,208} to propose laboratory experiments that minimize the uncertainty of estimated Monod kinetic parameters, these enhancements are largely inapplicable to traditional MRD microcosm experiments. For example, several studies^{204,209,210} have evaluated optimal batch reactor feeding profiles, but these are not relevant to typical MRD microcosm experiments that are conducted in small (i.e. less than 250 mL²⁰³) vessels and allowed to reach completion without further additions to the system. Other studies^{211–213} focused on optimal sampling times, but their results are not relevant to microcosms with sequential degradation. Most relevant design studies have focused on determining optimal substrate concentration to half-saturation constant parameter ratios. However, none have explored uncertainty reduction strategies for MRD subject to competitive inhibition.

Given these deficiencies in prior investigations, two MCEs with the following objectives are proposed for this paper: evaluate the precision and accuracy of parameter estimates derived from different experimental designs (Experiment 1), and test the coverage of different interval estimation methods (Experiment 2).

5.3 Methods

5.3.1 Microcosm MRD model formulation

Microcosm experiments considered herein are conducted in 160-mL vessels containing 125 mL of an aqueous phase (35 mL headspace), seeded with 1.00E8 cells/L of *Dehalococcoides mccartyi* (*Dhc*) and some initial concentration of *cis*-DCE. *Cis*-DCE is assumed to degrade via VC to ethene. One mL aqueous phase samples are extracted from the microcosms at regular intervals (20 in total) until ethene comprises 95% of aliphatic hydrocarbon (AH) compounds in the system.

The MRD rate, r_i^a , of a chlorinated AH (CAH) compound i in the aqueous phase (a) can be expressed according to Monod kinetics with competitive inhibition:

$$r_i^a = \frac{u_i X C_i^a}{K_i^s I_i + C_i^a} \quad (1)$$

where u_i is the maximum substrate utilization rate [mmol/cell/day], X is the concentration of dechlorinating bacteria [cell/L], C_i^a is the aqueous phase concentration [mM], K_i^s is the half-saturation constant [mM], and I_i is the inhibition coefficient.¹⁹⁵ Eq 1 assumes that electron donor is supplied in excess, consistent with typical microcosm experiments. Many different forms of competitive inhibition have been employed in the literature.¹⁹⁵ Here it is assumed that both CAH compounds are inhibitive to some extent^{160,172,214} and the inhibition coefficients I_i are:

$$I_{DCE} = 1 + \frac{C_{VC}^a}{K_{VC}^I} \quad (2)$$

$$I_{VC} = 1 + \frac{C_{DCE}^a}{K_{DCE}^I} \quad (3)$$

where K_i^I is the competitive inhibition constant. Given the range of competitive inhibition values tested (see Table 5.1), this model formulation will result in hypothetical microcosm systems exhibiting both strong and weak competition dynamics, enhancing the applicability range of model fitting experiment results.

When diffusive and sampling mass losses are incorporated in the model, the MRD microcosm mass balance equations can be expressed as²⁰³:

$$\frac{d}{dt}(C_i^a) = \frac{V^a R_i^a + \frac{C_i^a Q^a}{H_i R T} + k_i^{dl} C_i^a}{V^a + \frac{V_g}{H_i R T}} \quad (4)$$

$$\frac{d}{dt}(X) = \sum_j (Y_j r_j^a) - k_{Dhc}^b X \quad (5)$$

where t is time [days], i corresponds to *cis*-DCE, VC, or ethene, Q^a is a time-averaged aqueous phase sampling rate (approximating samples removed from the system) [L/day], H_i is the Henry's law coefficient [mmol/m³/Pa], R is the gas constant [J/K/mmol], T is temperature [K], k_i^{dl} is the effective diffusional mass loss rate coefficient [L/day], j corresponds to *cis*-DCE or VC, Y_j is the yield coefficient [cells/mmol], and k_{Dhc}^b is the endogenous cell decay rate [day⁻¹]. The overall reaction rate R_i^a for each AH is the sum of appropriate production and transformation rates, i.e., $R_{DCE}^a = -r_{DCE}^a$, $R_{VC}^a = r_{DCE}^a - r_{VC}^a$, and $R_{ETH}^a = r_{VC}^a$.

To produce synthetic microcosm data for a given Monod kinetic parameter set, Eqs 4 and 5 were numerically integrated^{182,215,216} and normally distributed random error was then added to generate concentration measurements. Here it was assumed that gas chromatography (GC) measurements of C_i^a have a coefficient of variation equal to 10%, and the standard deviation (σ) of X measurement error follows an empirical relationship estimated for previous quantitative polymerase chain reaction (qPCR) analysis¹³²:

$$\sigma_{GC} = 0.10 C_i^a \text{ [mM]} \quad (6)$$

$$\sigma_{qPCR} = 4.80(X)^{0.891} \text{ [cell/L]} \quad (7)$$

Since the magnitude of C_i^a and X vary over time, the measurement errors are heteroscedastic.

It is assumed that Monod kinetic parameters selected for each synthetic dataset do not change over the course of the microcosm experiment. Some studies^{161,217} have proposed designs intended to avoid changes in kinetic parameter values caused by long-term acclimation of the

microbial community to environments rich or poor in substrate. Such shifts in microbial community composition or characteristics are not considered herein.

Table 5.1: Model Parameters and Experimental Designs

Model Parameters			
Name	Fitting Parameter?	Value	Note
maximum substrate utilization rate (mmol/cell/day)			
u_{DCE}	yes	5.50E-14 to 3.20E-08	literature range ^{143,146,162,164,174,186,191,218}
u_{VC}	yes	8.00E-15 to 4.84E-09	literature range ^{137,143,146,159,162,164,174,186,218}
half-saturation constant (mM)			
K_{DCE}^S	yes	6.00E-04 to 3.83E-01	literature range ^{139,162,164,167,172,174,201,214,218–221}
K_{VC}^S	yes	7.00E-04 to 6.09E-01	literature range ^{139,162,164,167,172,174,214,218–221}
competitive inhibition constant (mM)			
K_{DCE}^I	yes	1.80E-04 to 9.51E-01	literature range ^{139,146,160,162,164,167,172,174,214,218,220}
K_{VC}^I	yes	5.45E-03 to 3.83E-01	literature range ^{160,172,214}
yield coefficient (cell/mmol)			
Y_{DCE}	yes	1.10E+09 to 1.43E+12	literature range ^{137,143,162,164,174,186,191,218}
Y_{VC}	yes	2.27E+09 to 6.88E+13	literature range ^{143,159,162,164,174,177,186,191,218,222}
endogenous cell decay rate ^a (day ⁻¹)			
k_{Dhc}^b	no	1.00E-2	typical literature value ^{164,195}
effective diffusional mass loss rate coefficient ^b (L/day)			
k_{DCE}^{dl}	no	5.00E-4	scaled to yield typical mass loss ²⁰³
k_{VC}^{dl}	no	1.68E-4	scaled to yield typical mass loss ²⁰³
k_{ETH}^{dl}	no	9.36E-6	scaled to yield typical mass loss ²⁰³
Experimental Designs			
	A	B	C
microcosms	3 (1 triplicate)	3	15 (5 triplicates)
initial <i>cis</i> -DCE concentrations [mM]	0.15	0.05, 0.20, and 0.35	0.01, 0.05, 0.20, 0.35, and 0.50
GC measurements per microcosm	20	20	20
qPCR measurements per microcosm	5	5	20
Experiment 1 trials ^c	360k	80k	105k
Experiment 2 RA trials	26k	32k	31k
Experiment 2 MCMC trials	0	96	0

Notes:

^a This parameter can be estimated from qPCR measurements made after all CAH compounds have been dechlorinated, so it was assumed to be known for the model fitting experiments.

^b These parameters can be estimated from abiotic control microcosms, so it was assumed to be known for the model fitting experiments.

^c Unequal numbers of trials are an artifact of the amount of computational resources available when each design was tested.

5.3.2 Parameter selection

Literature ranges for the eight fitting parameters considered in this paper (Table 5.1) span multiple orders of magnitude. This 8-dimensional “parameter space” is conceptualized as three nested zones in Figure 5.1b. Zone I is the outermost zone, which is an 8-dimensional rectangular prism comprising all points in parameter space within literature ranges. Initial testing indicated that only a small fraction of Zone I returns MRD model results with realistic dechlorination times (i.e., comparable to published MRD microcosm experiment results¹⁹⁵). The subspace of Zone I that returns realistic MRD model output is designated Zone II. If Monod kinetics adequately describes MRD, then the range (subspace) of parameter values that realistically describes bacterial MRD in nature likely resides in Zone II and is designated as Zone III. Very little consensus exists in the literature over the location of Zone III, so points in parameter space selected for model fitting experiments will be sampled from Zone II.

Hamiltonian Monte Carlo (HMC) was used to sample points from Zone II for generating synthetic microcosm datasets using three alternative designs (see Figure 5.1). HMC is an MCMC technique, like the Metropolis algorithm, where points are iteratively sampled in a Markov Chain, but the points returned by HMC are much less correlated.^{223,224} Three HMC chains (with 43.2k, 50.6k, and 50.8k samples each) were collected over 10 weeks on three 8-CPU computers following the methodology described in Section 5.5. This method of generating sets of parameters for model fitting experiments could be extended to other microbially mediated processes.

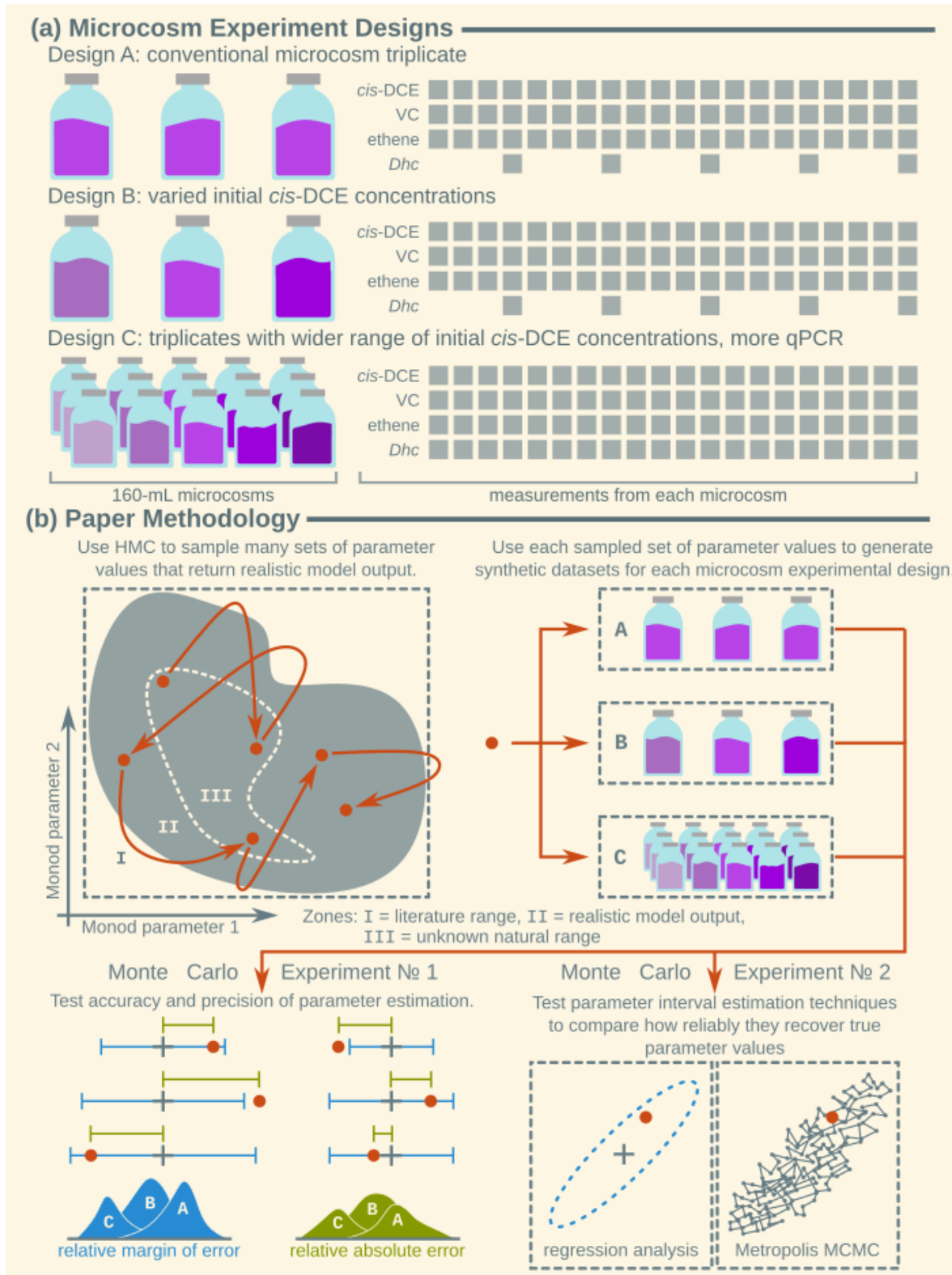


Figure 5.1: Microcosm Experiment Designs and Paper Methodology

5.3.3 Monod kinetic parameter estimation

For each model fitting experiment, one set of best-fit point estimates for the eight fitting parameters (Table 5.1) was determined by minimizing a weighted-least-squares objective function with differential evolution^{182,183}:

$$s^2(\theta) = \sum_{g=1}^G \sum_{m=1}^M \sum_{n=1}^N \frac{[\hat{y}_{gmn}(\theta) - y_{gmn}]^2}{[\hat{\sigma}_m(y_{gmn})]^2} \quad (8)$$

where θ is the vector of 8 fitting parameters, g corresponds to the microcosm, m corresponds to measured species (one bacterial and three chemical), n is the sample index, $\hat{y}_{gmn}(\theta)$ is the model concentration, y_{gmn} is the measured concentration, and $\hat{\sigma}_m$ is a function (Eq 6 or 7) approximating measurement error standard deviation. (Note that, in practice, the functional relationships between measurement value magnitude and measurement error standard deviation must be estimated by independently extracting and analyzing multiple samples from the same microcosm; measurements from replicate microcosms are inadequate for this purpose.)

For model fittings in Experiment 1 (see below) it was important that an objective function minimum be quickly located for tens of thousands of synthetic datasets. To complete those optimizations quickly, the bounds were set to one order of magnitude above and below the true parameter values used to generate the data. In contrast, model fittings in Experiment 2 could not be completed with any prior knowledge of the true parameter values. For those optimizations the bounds were set equal to the literature ranges in Table 5.1.

Two methods were used to determine interval estimates (see Figure 5.1b). The first method, RA, is commonly employed²⁰³ to assess parameter uncertainty in the MRD modeling literature. In this approach, derivatives of all model predictions with respect to the p fitting parameters are

estimated at their best-fit points, and the resulting Jacobian matrix is combined with measurement precision information to calculate a $p \times p$ covariance matrix (i.e. Cramér–Rao lower bound) from which p marginal confidence intervals are derived.²²⁵ Computational details are given in Section 5.6. The second method is the Metropolis algorithm²²⁶, an MCMC technique where multiple Markov chains take small random steps through the p -dimensional space of plausible parameter values, eventually yielding a large sample of fitting parameter values from which percentile-based interval estimates can be directly determined. MCMC techniques are frequently used in Bayesian statistical inference^{167,227}, but for experiments in this paper no prior distribution was employed; in practice, very little is known *a priori* about Monod kinetic parameter values. The likelihood derived from Eq 8 was used as the MCMC target distribution (see Section 5.7). For the Metropolis algorithm, the Hessian of the model fitting objective function (Eq 8) was used to initialize starting points for four Markov chains and to tune the proposal distribution so that it could more effectively sample the highest-likelihood region.²²⁷

5.3.4 MRD microcosm designs

For the purposes of this study a “dataset” is defined as the measurements from a collection of microcosms to which the model was fit simultaneously. A “design” describes how many microcosms were included in the dataset, their initial *cis*-DCE concentrations, and how many concentration measurements were taken. The designs (Figure 5.1a) took three general forms: Design A was intended to emulate MRD literature convention, i.e., a triplicate of microcosms with identical initial *cis*-DCE concentrations. Design B is a proposed alternative design, comprised of the same number of microcosms and measurements but with varying initial *cis*-DCE concentrations. (The rationale for varying initial *cis*-DCE concentrations follows from results of an MCE described in Section 5.8). Design C was intended to explore how reductions in

parameter estimation uncertainty realized by Design B could be further enhanced by increasing the number of microcosms and concentration measurements. See Table 5.1.

5.3.5 Monte Carlo Experiments 1 and 2

For Experiments 1 and 2 (see Figure 5.1b), each parameter space point tested was used to generate 5 unique synthetic datasets to which the model was independently fit (e.g., testing 72k points for Design A in Experiment 1 resulted in a total of 360k trials listed in Table 5.1). The objective of Experiment 1 was to assess best-fit parameter uncertainty using different microcosm experiment designs. This required many trials, so optimization bounds were set to one order of magnitude above and below true parameter values used to generate each dataset. Experiment 2 was intended to simulate the coverages researchers could expect different parameter interval estimation methods to yield, so optimization bounds were enlarged to encompass literature ranges in Table 5.1. Minimizing the objective function with larger optimization bounds required more computation than Experiment 1, so a smaller set of randomly selected parameter space points was tested. Best-fit point estimates from Experiment 1 were compared with true values to calculate error (i.e., accuracy), and RA was performed to calculate marginal confidence interval width (i.e., precision). RA was also used in Experiment 2 to calculate confidence intervals, but the result of each trial was eight Boolean values indicating which intervals contained true parameter values. Split- $\hat{R}$ and effective sample size (ESS) metrics^{228,229} confirmed the convergence of accuracy/precision measure percentiles reported for Experiment 1 and RA coverages reported for Experiment 2. The number of trials performed for Experiment 2 was sufficient to calculate precise confidence intervals for reported coverages.^{230,231} Based on results from Experiment 1, Design B was selected for further testing in Experiment 2 to see if MCMC resulted in more reliable parameter interval estimates than RA. The MCMC was more

computationally intensive than RA, so it was used to determine parameter interval estimates for 96 randomly selected Design B datasets.

Numerical model-fitting experiments were run on a 19-node computer cluster with 152 CPU cores for approximately three months. Each individual differential evolution optimization and RA takes a few minutes to run on a typical notebook computer, and the Metropolis MCMC takes about 24- 36 hours.

5.4 Results and Discussion

5.4.1 Monte Carlo Experiment 1: comparing microcosm experiment designs

Figure 5.2 summarizes relative margin of error (RMOE), i.e., the width of the 90% confidence interval, normalized by the best-fit point estimate, a measure of estimated parameter precision, for all parameters and designs. Here RMOE values greater than 1000% (10^1) indicate that the design is inadequate for even order-of-magnitude estimation. The RMOE for Design A (i.e., a conventional triplicate of microcosms) frequently exceeded 1000% for five parameters. Only two parameters (Y_{DCE} and Y_{VC}) had 75 percentiles below 100%.

RMOE for Design B (i.e., three microcosms with varied initial *cis*-DCE concentrations) was much lower than Design A, despite requiring an identical number of experimental measurements. Median RMOE for two parameters (K_{DCE}^S and K_{VC}^I) decreased an order of magnitude. On average, median RMOE decreased 70%. However, 95 percentiles for u_{VC} , K_{VC}^S , and K_{VC}^I were above 1000%.

Although RMOE for Design C (i.e., 5 microcosm triplicates with varied initial *cis*-DCE concentrations) was lower than Design B (a median RMOE decrease of 64% on average), none

of the parameters had an order of magnitude RMOE decrease. Median RMOE were all less than 10%. RMOE for u_{VC} , K_{VC}^S , and K_{VC}^I were still highest, with 95 percentiles near 1000%.

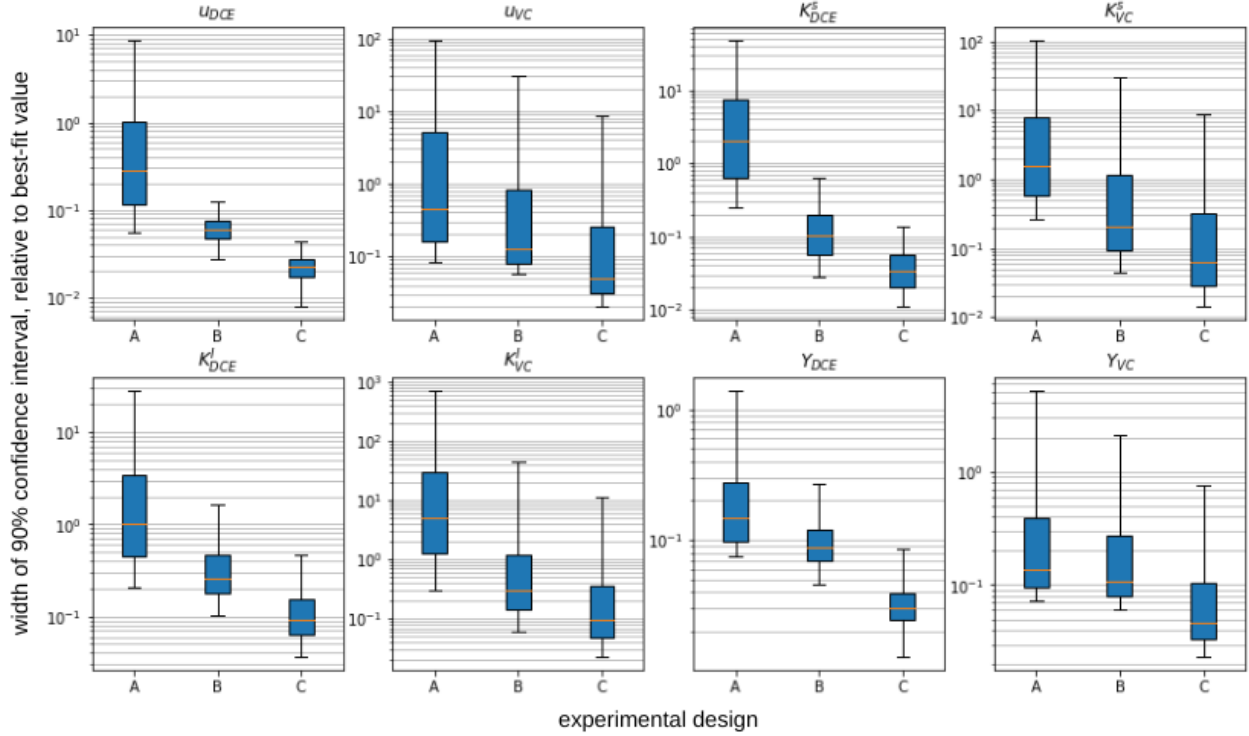


Figure 5.2: Parameter estimation precision (measured by relative margin of error, RMOE) of conventional microcosm experimental design (A) and improved experimental designs (B and C). Plotted percentile values (5, 25, 50, 75, and 95) summarize 0.55 million individual model fitting experiments.

To evaluate parameter estimation accuracy, relative absolute errors (RAE, absolute difference between best-fit point estimate and true value, normalized by true value) were calculated for all three designs (Figure 5.3). Upper optimization bounds for Experiment 1 model fits were 10 times the true value, so any boxes with 95 percentiles near 10^1 (i.e., five out of eight parameters for Design A) should be interpreted as extending beyond it. For Design A, Y_{DCE} was the only parameter that could be reliably estimated with less than 60% RAE. All other parameters frequently had estimation errors greater than 100%.

Compared to Design A, Design B had lower estimation errors for all parameters. The 95 percentile u_{DCE} RAE decreased an order of magnitude. Median K_{DCE}^S and K_{VC}^I RAE also decreased an order of magnitude. Overall, median RAE decreased an average of 61%.

Compared to Design B, Design C also had lower estimation errors for all parameters. However, the decreases in RAE were not as large as that for Designs A and B. On average, median RAE decreased 38%. Designs B and C both had higher RAE for the VC parameters than the *cis*-DCE parameters. This suggests that in sequential degradation scenarios, uncertainty in determining parameters for the first compound will propagate forward, increasing the uncertainty of parameters for the daughter compounds.

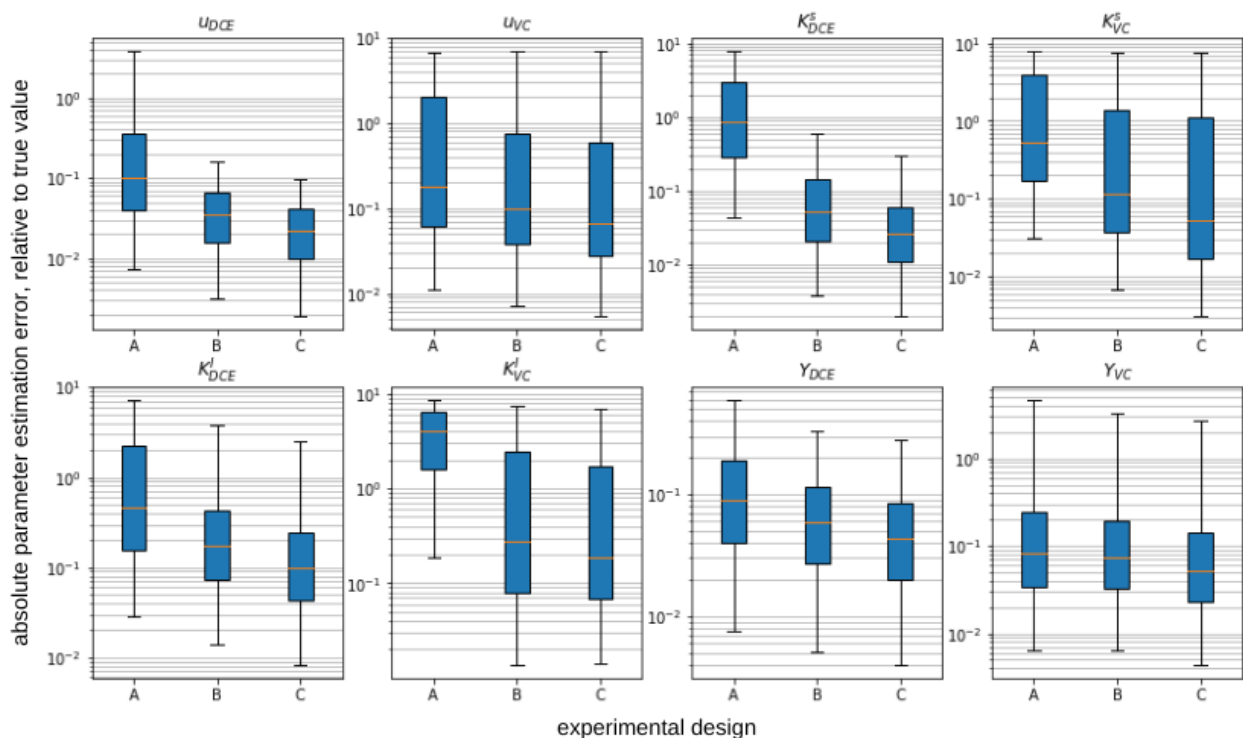


Figure 5.3: Parameter estimation accuracy of conventional microcosm experimental design (A) and improved experimental designs (B and C). Plotted percentile values (5, 25, 50, 75, and 95) summarize 0.55 million individual model fitting experiments.

To evaluate which sets of parameters frequently had collinearity problems and could not be estimated accurately, Belsley collinearity indexes²³² were calculated for Experiment 1 model-fitting results. Collinearity index values over 30 indicate that the parameters are strongly collinear and model fitting results will have uniqueness issues. Despite large relative differences in accuracy and precision, all designs suffered from strong collinearity problems; fewer than 0.1% of trials had condition indexes lower than 30. The top eight most frequently detected collinearities for each design are plotted in Figure 5.4. Note that the Belsley collinearity test will only reliably detect the strongest collinearities.²³³ Trials frequently had multiple detected collinearities among the fitting parameters.

Design A collinearities tended to involve more parameters, reflecting a stronger degree of collinearity. The average number of collinear parameters, weighted by the number of times each collinearity was detected, were 3.2, 2.4, and 2.3 for Designs A, B, and C, respectively. Design A also had a wider variety of detected collinearities, whereas Designs B and C produced a smaller set of collinearities that were detected more frequently (see histograms in Figure 5.4). Designs B and C shared seven of the eight most frequently detected collinearities, suggesting that increasing the number of measurements and microcosms did not fundamentally alter the nature of the inverse problem. That is, adding more data did not change the geometry of the highest-likelihood region in parameter space nor change which sets of parameters tended to be collinear.

Previous work^{153,184,201} on single-substrate Monod kinetics without inhibition has suggested that a higher initial substrate concentration to K_i^S ratio results in greater parameter identifiability. All Design A datasets in this MCE had the same initial *cis*-DCE concentration (0.15 mM), but half-saturation constants K_{DCE}^S and K_{VC}^S varied across the literature ranges in Table 5.1. This enabled an evaluation of whether this purported relationship manifested itself in this paper's results.

Figures 5.7 and 5.8 in Section 5.9 present plots of K_i^s versus the collinearity index for fitting parameter collinearities involving u_i and K_i^s . If a higher ratio resulted in greater identifiability, that would be exhibited as a negative correlation in Figures 5.7 and 5.8. No trend is apparent, indicating that observations regarding this ratio in prior work are not applicable to sequential Monod degradation with competitive inhibition.

Section 5.8 details a supplemental MCE that assessed how the initial *cis*-DCE concentration of a microcosm triplicate influences the value of the dataset for kinetic parameter estimation. In contrast to the aforementioned prior work, results from this MCE indicate that a lower initial *cis*-DCE concentration tends to aid estimation of u_i and K_i^s for the more complex model considered in this paper.

Overall, Design A had the highest degree of collinearity, and Designs B and C had relatively lower collinearity. The average median condition index value, weighted by the number of times each collinearity was detected, were 33506, 5438, and 2844 for Designs A, B, and C, respectively. These averaged values indicate that model-fitting results for Design B had markedly less collinearity than Design A, but greatly increasing the amount of laboratory measurements in Design C resulted in less dramatic improvement.

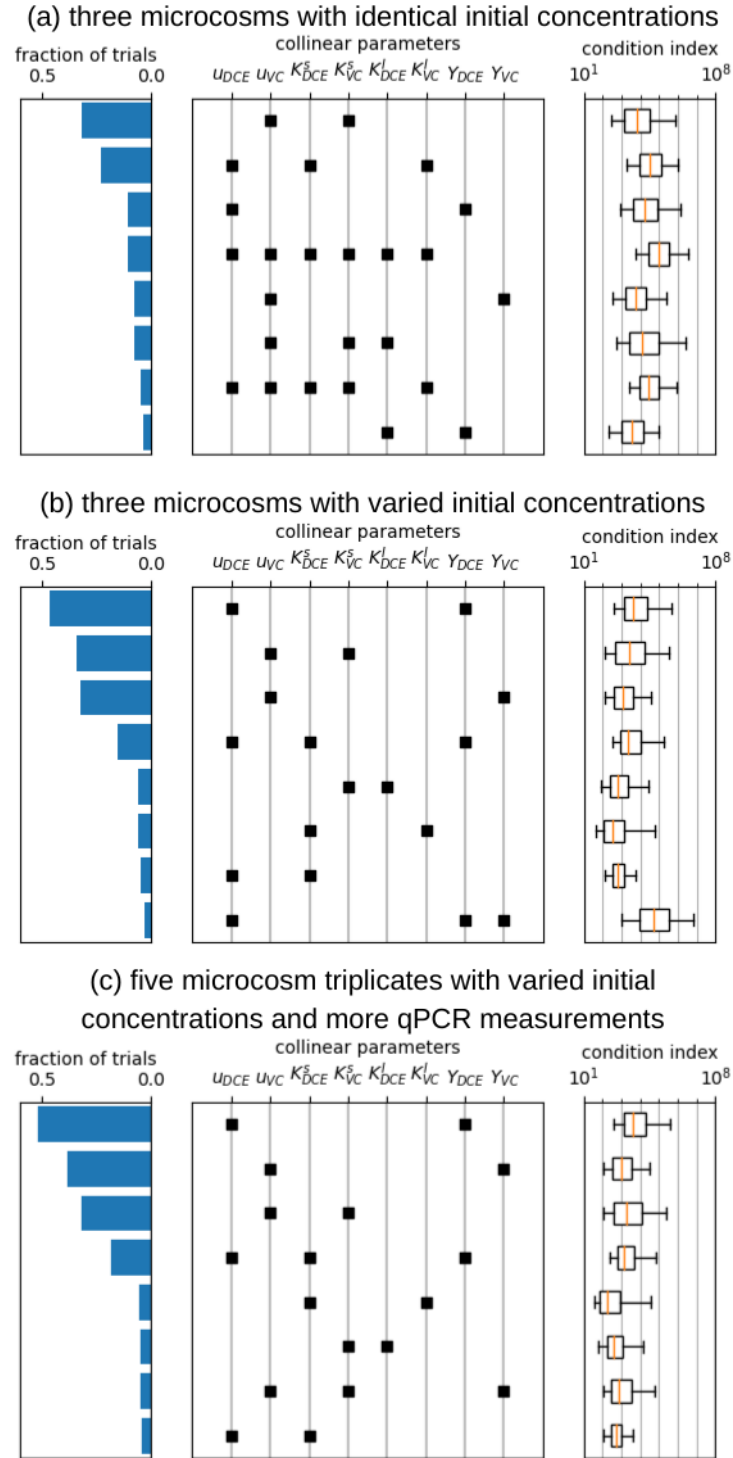


Figure 5.4: Collinearities detected in model fitting results for different microcosm experiment designs. The parameters in each collinearity group are indicated with squares in the center pane, the fraction of datasets with that collinearity are plotted in the left pane, and the distribution of collinearity index values associated with each collinearity are summarized with boxplots in the right pane.

5.4.2 Monte Carlo Experiment 2: evaluation of interval estimation techniques

RA results for Experiment 2 are summarized in Table 5.2. Coverage (i.e., the fraction of confidence intervals that contained the true parameter value) was well below the nominal confidence level of 90% for all three designs (95% margins of error for the estimated coverages were all less than 1%). Despite its widespread use in the MRD literature²⁰³, these results indicate that RA should not be applied for simultaneous estimation of kinetic parameters for sequential Monod processes.

As an alternative to RA, MCMC with the Metropolis algorithm was tested to see how reliably its interval estimates contained true parameter values. MCMC is much more computationally intensive than RA, so a smaller quantity of model fitting experiments could be performed. Approximately 100 synthetic datasets generated with Design B were analyzed with MCMC. Here Design B was selected over Designs A and C, based upon the poor accuracy and precision of the former and the excessive amount of lab work required to implement the latter. MCMC parameter interval estimation results are summarized in Table 5.2 and Figure 5.5.

A measure typically recommended to assess MCMC convergence is the split- $\hat{R}$ statistic²²⁸, which decreases to unity as the Markov chains begin to return a representative sample of the target distribution (which in this context is the maximum likelihood region in parameter space for a microcosm MRD model fit). A split- $\hat{R}$ statistic was calculated for each fitting parameter, p , and plotted in Figure 5.5 as the mean split- $\hat{R}$, biased by unity, to facilitate plotting on a log-scale:

$$\bar{R} = \left(\frac{1}{P} \sum_{p=1}^P \hat{R}_p \right) - 1 \quad (9)$$

Line colors in Figure 5.5 indicate how many true parameter values were enclosed by the corresponding MCMC interval estimate (determined by finding the 5 and 95 percentile of values sampled by the Metropolis algorithm) at that iteration. A few MCMC analyses had better coverage as iterations progressed, but most had approximately the same number of recovered parameters over the course of the analysis. The Hessian of the model-fitting objective function was used to randomly sample starting points for the four MCMC chains. If the differential evolution optimizer returned a point in parameter space that was near the point representing true parameter values, then the MCMC chains tended to start and then converge in the neighborhood surrounding that true point, with low or decreasing $\bar{R}$ values as iterations progressed. If the differential evolution point estimates were far from true values (gray lines), then the MCMC chains did not approach or reach the region surrounding the true point with low associated $\bar{R}$ values. These results indicate that interval estimates from a converged MCMC analysis can be trusted to contain true values for several of the parameters. An $\bar{R}$ less than 0.05 appears to be an adequate empirical heuristic for MCMC convergence.

The last two columns of Table 5.2 summarize results from 16 MCMC trials that achieved an $\bar{R}$ less than 0.05. Median RMOE from the MCMC trials closely matches the distribution of RMOE values plotted in Figure 5.2. Coverage values are still slightly lower than the nominal confidence level of 90%, but higher than what RA achieved for Designs B and C. A hypothesis test¹⁸⁹ constructed with the chi-squared statistic (see Section 5.10) suggests that measured coverage deviates from the nominal confidence level enough to indicate that 90% is not being achieved. Accurately estimating tail quantiles of a target distribution requires more MCMC iterations than estimating less extreme quantiles.²²³ If estimating precise tail quantiles is important for statistical inference (e.g., comparing the performance of two similar MRD inoculums, or comparing the

performance of an inoculum in slightly different conditions), then the target $\bar{R}$ should be lower than 0.05. The ESS metric can also be used to test if tail quantiles have been accurately estimated.²²⁸

The coverage values listed in Table 5.2 should not be misinterpreted as indicating that Design A is superior to the other designs. As described in the previous section and plotted in Figures 5.2 and 5.3, Designs A, B, and C had progressively better accuracy (i.e., smaller RAE) and precision (i.e., smaller RMOE). Design A had very poor precision, with confidence intervals that were frequently too large even for order-of-magnitude estimation. Varying the initial *cis*-DCE concentrations in Design B resulted in large precision improvement, and increasing the amount of data in Design C resulted in equivalent additional precision improvement. In contrast, accuracy improvement between Design A and B was larger than the accuracy improvement between B and C. In other words, the size of the confidence intervals greatly decreased, but that improvement was not fully matched by improvement in how closely the corresponding best-fit point estimates approximated the true parameter values. Expanding optimization bounds to literature ranges for all Experiment 2 model fits only exacerbated these accuracy issues, with median RAE, averaged across the eight fitting parameters, increasing by 244%, 184%, and 167% over Experiment 1 for Designs A, B, and C, respectively.

As described previously, Designs B and C had a relatively much lower degree of fitting parameter collinearity than Design A, but all designs still suffered from high collinearity and associated nonuniqueness problems. The differential evolution optimizer utilized in this paper is a heuristic algorithm that begins the optimization process with a population of potential candidate solutions scattered throughout parameter space. Compared to quasi-Newton optimization algorithms like L-BFGS that start from one point in parameter space and use

estimates of the Hessian to iterate towards a minimum, differential evolution may take longer to find an optimal solution but is less likely to get stuck in a local minimum.^{234,235} Initial testing (results not shown) indicated that differential evolution consistently outperformed the L-BFGS algorithm for the inverse problem considered in this paper. Accordingly, any RA of sequential MRD model fitting results in the literature that uses a best-fit point supplied by a quasi-Newton optimizer likely has even worse coverage than the results in Table 5.2. In particular, the popular spreadsheet-based method¹⁵⁸, which already has parameter estimation errors caused by ignoring experimental mass loss²⁰³, is wholly inadequate for the task.

Table 5.2: Comparing Reliability of Regression Analysis and MCMC

Parameter	Regression Analysis Coverage			MCMC with Design B	
	Design A	Design B	Design C	Coverage	median RMOE
u_{DCE}	69%	55%	38%	75%	6%
u_{VC}	72%	71%	54%	94%	14%
K_{DCE}^S	73%	62%	47%	81%	7%
K_{VC}^S	75%	77%	65%	88%	16%
K_{DCE}^I	65%	57%	40%	75%	24%
K_{VC}^I	77%	69%	42%	75%	17%
Y_{DCE}	49%	49%	27%	63%	8%
Y_{VC}	51%	53%	36%	88%	10%

Notes:

- Tabulated values are for Experiment 2 results.
- Coverage and relative margin of error (RMOE) values for MCMC are limited to the 16 model fitting experiments with a mean split- $\hat{R}$ statistic less than the recommended threshold of 1.05. Refer to Figure 5.2 for precise estimates of the RMOE that each experimental design is likely to yield.

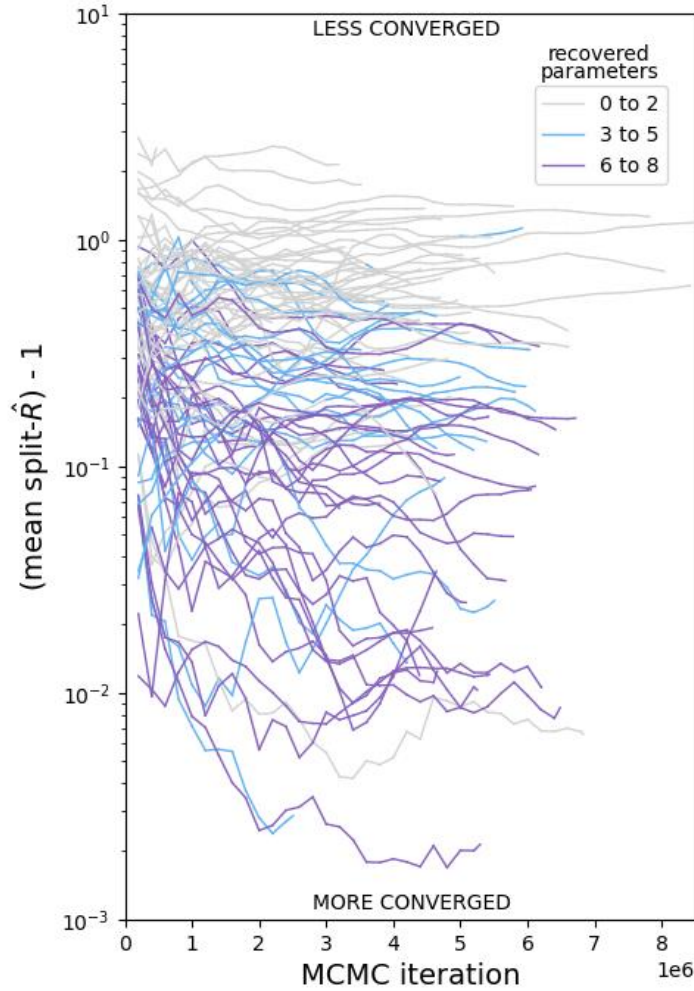


Figure 5.5: Convergence and coverage of 96 MCMC parameter uncertainty analyses.

5.4.3 Recommended methodology for estimating sequential Monod parameters

The above results indicate that, for more reliable parameter estimation, an improved microcosm design would start with varied initial CAH compound concentrations, as employed in Design B. Results from Design C model fitting experiments indicate that augmenting the dataset with microcosm replicates and additional qPCR measurements will further decrease parameter uncertainty. After minimizing the model fitting objective function (Eq 8) with differential evolution, it is recommended that the best-fit point be used to initialize an MCMC parameter uncertainty analysis. If MCMC fails to achieve an $\bar{R}$ less than 0.05, then the differential

evolution optimization should be restarted with several different random seeds, and the MCMC should be repeated for each resulting best-fit point.

If that procedure fails to yield MCMC convergence, then the fitting parameters may be intractably collinear, and additional laboratory experiments are needed. In this scenario, a Belsley collinearity test will indicate which parameters are collinear and thus what sort of experiments will resolve the nonuniqueness. For instance, if parameters for the daughter CAH compounds are collinear, then a separate set of microcosm experiments initialized with that compound are recommended. If the competitive inhibition constants are collinear with other parameters, then a set of experiments designed to directly measure competitive inhibition effects¹⁶⁰ will be helpful.

RA techniques are parsimonious in their use of computational resources, but not in the assumptions required for their valid application.¹⁵⁵ The results in this study have demonstrated that continued use of these methods to analyze conventional microcosm experiment data is likely to result in high error and extreme uncertainty. Application of the design and interval estimation techniques described herein are expected to narrow literature ranges of Monod parameters and increase confidence in field-scale model predictions of MRD remediation performance. The Metropolis algorithm is computationally intensive, but it returns a strong signal (low split- $\hat{R}$ values) when its results can be trusted. Whether other MCMC algorithms or uncertainty quantification techniques can address this inverse problem more efficiently or effectively is a worthwhile topic for further research.

5.5 Definition of Target Distribution for Hamiltonian Monte Carlo

The model fitting experiments in this paper required generation of several thousand sets of Monod kinetic parameter values that resulted in realistic simulated microcosm data. To generate

these parameter sets, a Hamiltonian Monte Carlo (HMC) algorithm was used. Like other Markov Chain Monte Carlo (MCMC) methods, the purpose of HMC is to sample points from some probability distribution. This “target distribution” is usually derived from some multivariate model-fitting objective function, and the sampled points are analyzed (e.g., determining the medians, correlation coefficients, and 5-to-95 percentile range) to estimate fitting parameter values and evaluate uncertainty. However, in this context we define the target distribution to be a subset of the 8-dimensional Monod kinetic fitting parameter space that meets our numerical measures of realism. The realism criteria that these numerical measures assessed were:

1. All parameters must be within literature bounds (see Table 5.1).
2. The time t_T required for 95 percent dechlorination in a microcosm initialized with *cis*-DCE and proceeding to ethene should fall in the approximate range of 10 to 280 days.
3. The time t_{VC} required for 95 percent dechlorination in a system initialized with VC should be between approximately 5 and 140 days.
4. The time t_{DCE} required for 95 percent dechlorination of *cis*-DCE in a hypothetical microcosm where VC is not allowed to accumulate should be between approximately 5 and 140 days.

These numerical measures were developed based on a survey of the literature. A bump function²³⁶ was defined for each of the realism criteria above such that a value of zero is returned for input outside of the specified range (i.e. the literature ranges for criterion 1 and dechlorination time ranges for criteria 2-4), a value between zero and one is returned for input values near the range boundaries, and a value of one is returned for input values well within the range.

A continuously differentiable bump function $g(t)$ which is unity on $\{t \in \mathbb{R} \mid c - a < t < c + a\}$ and has support on $\{t \in \mathbb{R} \mid c - b < t < c + b\}$ can be constructed as follows.

$$f(t) = \begin{cases} \exp(-1/t) & \text{for } t > 0 \\ 0 & \text{for } t \leq 0 \end{cases} \quad (10)$$

$$h(t) = \frac{f(t)}{f(t) + f(1-t)} \quad (11)$$

$$k(t) = 1 - h\left(\frac{t - a^2}{b^2 - a^2}\right) \quad (12)$$

$$g(t) = k(t - c) \quad (13)$$

For each parameter θ_i , define a bump function $g_{L,i}(\theta_i)$ that has support over the corresponding range of values cited in the literature. A combined bump function for criterion 1 is then:

$$g_L(\theta) = \prod_{i=1}^m g_{L,i}(\theta_i) \quad (14)$$

If g_T , g_{VC} , and g_{DCE} are the bump functions for criteria 2, 3, and 4, respectively, then the target distribution for HMC is given by:

$$\pi(\theta) = \frac{1}{Z} g_L(\theta) g_T(t_T(\theta)) g_{DCE}(t_{DCE}(\theta)) g_{VC}(t_{VC}(\theta)) \quad (15)$$

where Z is an unknown normalizing constant.

5.6 Regression Analysis

It is assumed¹⁵⁵ that the measured value of dependent variable m (i.e., C_t^a or X) at time t_n is equal to the best-fit model prediction plus measurement error:

$$y_{gmn} = \hat{y}_{gmn}(\hat{\theta}) + e_{gmn} \quad (16)$$

where $\hat{\theta}$ is the vector of $P = 8$ best-fit point values for the model parameters. For notational convenience, define a subscript $u = 1, 2, \dots, U$ that indexes over all measurements.

$$y_u = \hat{y}_u(\hat{\theta}) + e_u \quad (17)$$

Calculate a $P \times P$ matrix A :

$$(A)_{pq} = \sum_{u=1}^U \left[\left(\frac{1}{\hat{\sigma}_u(y_u)^2} \right) \left(\frac{\partial \hat{y}_u(\hat{\theta})}{\partial \theta_p} \frac{\partial \hat{y}_u(\hat{\theta})}{\partial \theta_q} \right) \right] \quad (18)$$

where $q = 1, 2, \dots, P$, $\hat{\sigma}_u(y_u)$ is the estimated measurement error standard deviation (Equation 6 or 7), and the partial derivatives are estimated with finite differences.²³⁷ The Cramér–Rao lower bound is then given by the matrix inverse of A .²²⁵ A marginal confidence interval for fitting parameter p is given by:

$$\hat{\theta}_p \pm t(U - P, \alpha/2) \left[\frac{s^2(\hat{\theta})}{U - P} (A^{-1})_{pp} \right]^{\frac{1}{2}} \quad (19)$$

where $t(U - P, \alpha/2)$ refers to the t-distribution value with $U - P$ degrees of freedom and tail probability $\alpha/2$, and $s^2(\hat{\theta})$ is the best-fit objective function value (Equation 8).²⁰⁶

5.7 Definition of Target Distribution for Metropolis Monte Carlo

The target distribution for the Metropolis algorithm used in this paper is the likelihood of the eight fitting parameters θ , i.e., the probability of θ conditioned on the measurements y_u from a synthetic dataset.

$$\mathcal{L}(\theta) = \prod_{u=1}^U \frac{1}{\hat{\sigma}_u(y_u) \sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{y_u - \hat{y}_u(\theta)}{\hat{\sigma}_u(y_u)} \right)^2 \right] \quad (20)$$

5.8 Supplemental Monte Carlo Experiment: Detecting Monod Kinetics and Competitive Inhibition

5.8.1 Experiment Method

The objective of this Monte Carlo experiment was to evaluate if the conventional triplicate of microcosms reliably produces data that exhibits both Monod kinetics and competitive inhibition. Each point in parameter space tested (more than 36k in total) was used to generate five synthetic datasets. Each dataset had a triplicate of microcosms at one of five initial *cis*-DCE concentrations: 0.01, 0.05, 0.20, 0.35, and 0.50 mM. Three MRD models were fit to each synthetic dataset:

1. Monod kinetics with competitive inhibition (i.e., the model described in the manuscript that was used to generate all synthetic datasets)
2. Monod kinetics without competitive inhibition (i.e., Model 1 with K_{DCE}^{inh} and K_{VC}^{inh} fixed to 1E4)
3. First-order kinetics (i.e., Model 2 with K_{DCE}^s and K_{VC}^s fixed to 1E4).

Models 1 through 3 can be conceptualized as increasingly constrained versions of the same model, with eight, six, and four fitting parameters, respectively. Since we know Model 1 was used to generate the synthetic datasets, the objective of this Monte Carlo Experiment is to evaluate if the conventional triplicate of microcosms (i.e., Design A) can serve as a sufficiently strong source of information to reveal that the process is governed by Monod kinetics with inhibition. Our objective is not to evaluate whether Models 2 and 3 (i.e., the *wrong* models) can also produce a good model fit to laboratory microcosm data. This problematic curve-fitting philosophy is evident in other work¹⁹⁹ that generated synthetic microcosm datasets with a Monod kinetics biodegradation model and then fit various simpler models to see which produced the

best fit. Achieving good model fit is ancillary to ascertaining what underlying model produced the data; parameters estimated from a good fit with the wrong model are of questionable value in contexts outside of that particular laboratory experiment (e.g., field-scale modeling).

Model 1 incorporates two complex dynamics in its behavior that are successively stripped away by Models 2 and 3: competitive inhibition and the mixed-order Monod regime. The degree to which each dynamic is exhibited in a dataset is partially a function of the initial *cis*-DCE concentration, and a clear exhibition of each dynamic is a prerequisite for (but not a guarantee of) estimating the associated kinetic parameters with limited uncertainty. For example, if the initial *cis*-DCE concentration is very low, then unless the true competitive inhibition constants K_i^I are also very low no competitive inhibition will be evident in the dataset and the inverse problem of estimating K_i^I is impossible. If the initial *cis*-DCE concentration is higher than some threshold such that datasets clearly exhibit competitive inhibition, estimating K_i^I may be possible but those estimates may be highly uncertain if the inverse problem is still ill-posed for other reasons.

After fitting the models, the minimum objective function values (Equation 8) were then compared via a Wilks test, a tool for determining whether added model complexity is statistically justified by a better model fit.²²⁵ A large quantity of model-fitting trials were required, so all model fittings had optimization bounds set to an order of magnitude above and below the true parameter values used to generate the data. For the weighted-least-squares regression scheme employed in this paper, the Wilks test defines the log-likelihood ratio as:

$$\lambda = 2(s_{full}^2 - s_{const}^2) \quad (21)$$

where s_{full}^2 and s_{const}^2 are the objective function values from the full and constrained models, and the constrained model is derived from the full model by specifying p' of the p fitting parameters (e.g., Model 2 is derived from Model 1 by fixing the $p' = 2$ competitive inhibition constants to 1E4). Under the null hypothesis, that the parameters are constrained as specified, the log-likelihood ratio λ is asymptotically chi-squared distributed with p' degrees of freedom. That is, if λ^* is the value found in a chi-squared distribution table with p' degrees of freedom and tail probability α , then the constrained version of the model can be rejected with at least confidence level $1 - \alpha$ if $\lambda > \lambda^*$.

Using the methodology described above, two log-likelihood ratios were calculated for each synthetic dataset: one comparing model fits 1 and 2 and another comparing model fits 1 and 3. Model 1 has more fitting parameters than Models 2 and 3, so it should have a lower objective function value. However, the simpler Models 2 and 3 can only be rejected if that difference in objective function values satisfies the Wilks test.

5.8.2 Experiment results

The model with competitive inhibition outperformed (i.e., achieved a lower model-fitting objective function value than) the no-inhibition model in a majority of all trials, but the degree of that outperformance was often not great enough to prove inhibition was occurring. Figure 5.6a displays the fraction of Monte Carlo trials where the Wilks test correctly rejected the model without inhibition (green) versus the fraction of tests with an inconclusive result (orange and red). The frequency with which data from a microcosm triplicate confirmed the existence of the competitive inhibition dynamic was positively correlated with the initial cis-DCE concentration. At the highest concentration tested (0.50 mM) the no-inhibition model was rejected in 92 percent of trials, near the nominal Wilks test confidence level of 95 percent. The strength of the

competitive inhibition dynamic is dependent on the interplay between CAH compound concentrations and the magnitude of true competitive inhibition constants K_i^I , but these results suggest that even weak inhibition can be detected with a conventional microcosm triplicate if the initial CAH concentrations are high enough. (Whether that data can then be used to reliably estimate K_i^I is examined in Monte Carlo Experiments 1 and 2.)

In contrast to results for the model with competitive inhibition, data from microcosm triplicates with a lower initial *cis*-DCE concentration tended to provide stronger evidence for correctly rejecting first-order kinetics in favor of Monod kinetics (Figure 5.6b). It was only for microcosms with the lowest initial *cis*-DCE concentration tested (0.01 mM) that the first-order model rejection rate approached the nominal Wilks test confidence level of 95 percent. This result differs from previous work^{153,184,201} on single-substrate Monod kinetics without inhibition, which found that a higher initial substrate concentration to K_i^S ratio resulted in greater parameter identifiability. This difference suggests that conclusions regarding the best experimental design for these simpler models cannot be directly applied to the more complex model formulations utilized in most MRD studies. (The purported relationship between parameter identifiability and the initial CAH concentration to K_i^S ratio is examined further in Monte Carlo Experiment 1.) As detailed in the introduction, previous numerical studies have had a small number of trials and failed to examine a large quantity of points in parameter space. Therefore, these studies also could have suffered from a lack of statistical power and parameter space selection bias.

An inability to distinguish between Monod kinetics and first-order kinetics suggests collinearity between u_i and K_i^S .¹⁹⁵ Some authors have suggested that fitting the ratio u_i/K_i^S instead of the two parameters independently can resolve that nonuniqueness problem.¹⁵¹ If fitting such a ratio results in a unique model fit without introducing additional artificial constraints on the

optimization problem, then that indicates u_i and K_i^S are correlated. That is, values that satisfy the linear function $u_i = \beta K_i^S$ result in equivalently good model fit, where β is a constant. Such a scenario is plausible for the degradation of one compound via Michaelis–Menten kinetics¹⁹⁵, but the model under consideration here contains many more fitting parameters; any collinearity that emerges could involve a much more complex equation describing a linear subspace of the 8-dimensional parameter space. Further analysis of these model fitting experiments suggests that the collinearity relationships are complex: comparing the ratio u_i/K_i^S calculated using estimated parameters to the ratio calculated using true parameters suggested that fitting this ratio would result in estimation errors equivalent to fitting the parameters separately. Furthermore, no relationship was discernable between these two parameters and the first-order model rate coefficients.

In summary, there was no overlapping initial *cis*-DCE concentration at which both Wilks tests were effective. This suggests that any experimental design that utilizes microcosm replicates with the same initial CAH concentration will likely suffer from parameter identification issues. Results from Monte Carlo Experiment 1 demonstrate how fitting the model simultaneously to microcosms with varied initial CAH concentrations can greatly improve parameter identifiability.

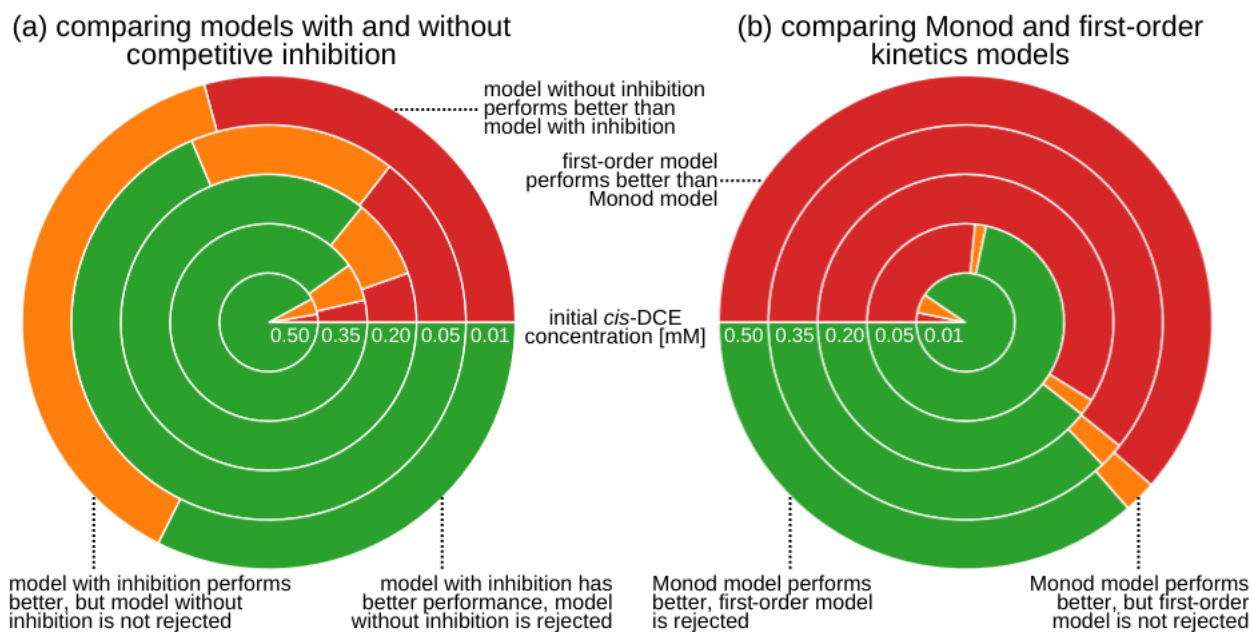


Figure 5.6: Relationship between initial *cis*-DCE concentration and ability to detect competitive inhibition and Monod kinetics with a Wilks test. Note that achieving better model performance is defined as obtaining a lower model-fitting objective function value. Note that the ordering of initial *cis*-DCE concentrations is flipped between (a) and (b) to facilitate plot labeling.

5.9 Collinearity versus the Ratio between CAH Concentration and the Half-Saturation Constant

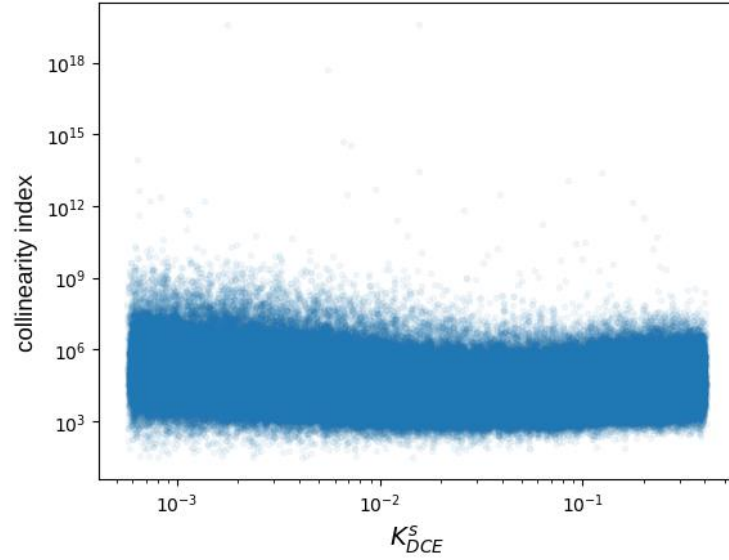


Figure 5.7: The collinearity index of all fitting parameter collinearities involving both u_{DCE} and K_{DCE}^S versus the true K_{DCE}^S value for all Design A datasets in Monte Carlo Experiment 1.

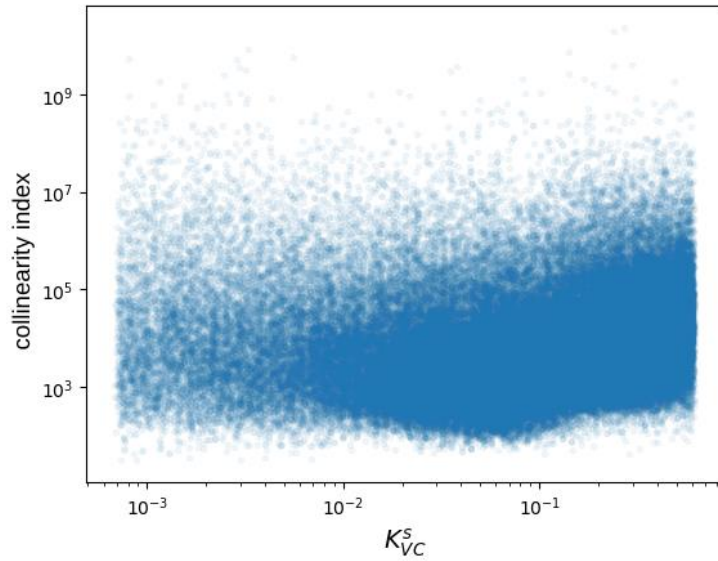


Figure 5.8: The collinearity index of all u_{VC} and K_{VC}^S collinearities versus the true K_{VC}^S value for all Design A datasets in Monte Carlo Experiment 1.

5.10 Chi-Squared Test for Evaluating MCMC Coverage

Among 16 MCMC trials with 8 fitting parameters, 102 true parameter values were observed to be within the prediction intervals, and 26 were outside. If the confidence level of the prediction intervals is 90 percent, then the expected value (i.e., the average value over many sets of 16 trials) of the number of prediction intervals that contain true parameter values is approximately 115, and the corresponding number of prediction intervals that do not contain the true parameter is 13. This deviation results in a chi-squared statistic of 15.1. The chi-squared statistic in this context approximately follows²³⁸ a chi-squared distribution with 1 degrees of freedom, but the probability of such a high value is less than 1 percent, rejecting the null hypothesis that the theoretical confidence level is being achieved.

5.11 Acknowledgements

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6 Conclusions and Recommendations for Future Work

The inverse problem of estimating Monod kinetic parameters from laboratory microcosm or batch reactor data is ill-posed, and nothing presented in this dissertation will likely change that: simultaneously estimating multiple Monod kinetic parameters using data from a sequential MRD experiment will likely always result in fitting parameters obscured by high collinearity.

However, if one follows the practices and methodology outlined in the preceding chapters, then parameter estimation uncertainty can probably be reduced to a level adequate for many statistical inference objectives.

Some of these recommended practices may moderately increase the amount of time, expense, and computation required to complete the parameter estimation process. For example, performing laboratory experiments with varied initial CAH concentrations means that each experiment will have to be sampled at a different interval, as opposed to current practice where everything can be sampled together once every few days. Quantifying the measurement precision of GC and qPCR analysis for weighted least squares regression requires the additional expense of independently extracting and analyzing replicate samples. The task of determining parameter interval estimates with MCMC will need at least a day of computation and is best performed on a computer cluster. Compared to the time and effort it typically takes to conduct MRD batch reactor or microcosm experiments, however, these extra considerations require fewer resources. And as demonstrated in the previous chapter, opting to follow conventional experimental design and parameter estimation methodology will likely result in fitting parameter intervals that are unsuitable for order-of-magnitude estimation.

If MRD modeling results are to be used outside of academia, then researchers need to form a better consensus over parameter ranges for specific Monod kinetics parameters. In the MRD

literature, describing the parameter estimation methodology is often relegated to a brief mention of the software that was applied. The challenging nature of this inverse problem, however, demands that great care be used to ensure that assumptions inherent in both the physical and statistical models are valid. Without such care, it is likely that the published parameter bounds in the literature will only expand, making this work of little use to the practitioner, who needs accurate and precise parameter estimates to: (1) predict the outcome of bioremediation with Monod-kinetics models, (2) compare the performance of different bioaugmentation inoculums, and (3) evaluate how different subsurface conditions may affect MRD.

Primary conclusions to be drawn from results presented in the preceding chapters are:

- Failing to account for experimental mass loss in the MRD model will likely result in large additional parameter estimation error, even if the number of estimated parameters is small. By extension, any other epistemic model error that adds a consistent bias to response variable measurements (e.g., underestimation of the initial *Dhc* concentration caused by insufficient inoculum qPCR analysis, overestimation of ethene concentrations caused by coelution with some other compound during GC analysis) will likely result in large parameter estimation errors. Statistical models inherent in the regression schemes utilized by this dissertation (ordinary least squares and weighted least squares) all assume model residuals are uncorrelated. Violating that assumption can impart difficult-to-predict bias on estimated parameter values.
- For most batch reactor or microcosm systems the experimental mass loss can be adequately approximated with the first-order model formulation presented in Chapter 2. For longer duration experiments the more sophisticated formulation presented in Chapter 3 will probably be necessary. In both scenarios it is recommended that abiotic

microcosms or batch reactors be initialized with all CAH compounds and ethene so that mass loss parameters for each compound can be estimated directly instead of using correlations.

- The laboratory analytical methods GC and qPCR vary greatly in terms of the magnitude of measured values and the structure of their respective measurement errors. Any model-fitting function based on these measurements should take these differences into account. If statistical inference is to be performed (e.g., calculating and comparing estimated parameter confidence intervals) then a model-fitting function that maximizes the likelihood should be used.
- Utilizing a model-fitting function that maximizes the likelihood requires knowing how measurement error standard deviation behaves as a function of the measurement magnitude, and characterizing measurement errors in this way requires independently extracting and analyzing several sets of replicate samples from the same batch reactor or microcosm. The following data are generally inadequate for this task: (1) samples extracted from replicate microcosms or batch reactors at the same time, (2) GC calibration curve measurements, (3) multiple qPCR instrument gene counts that have been made of DNA derived from one prepared sample.
- Literature values for the Monod kinetic parameters should be used with caution because of the lack of consensus over their typical ranges.
- Forms of competitive inhibition posited in the literature should be used with caution because of the lack of consensus over which compounds inhibit the MRD of other CAH compounds. If a Wilks test (see Chapter 5) does not justify the inclusion of competitive

inhibition in the model, then it is likely that no competitive inhibition constants can be estimated.

- The MRD model should be fit simultaneously to all data, not to mean measurements from replicate microcosms or batch reactors.
- Three microcosms or batch reactors with varied initial CAH concentrations will likely have order-of-magnitude better parameter estimation precision and accuracy than the conventional triplicate of experiments.
- Classical regression analysis (such as that employed by the MATLAB function “nlparci”) should probably not be used to determine parameter interval estimates for reasons elucidated in Chapter 5. The Smith et al. spreadsheet technique is also likely inadequate because in addition to using regression analysis it also assumes no experimental mass loss occurs.
- Compared to differential evolution, quasi-Newton optimization algorithms like L-BFGS that start from one point in parameter space and use estimates of the Hessian to iterate towards a minimum are more likely to return best-fit point estimates farther from true parameter values.
- MCMC with a simple Metropolis algorithm can be used to confirm that a best-fit point returned by differential evolution is near true parameter values. If the MCMC converges with mean split- $\hat{R}$ less than 1.05, then its interval estimates can likely be trusted.
- For batch reactor experiments, the inhibition of *cis*-DCE and VC MRD caused by PFAA is well described by a log-logistic dose-response model.

Based on the results presented in this dissertation, questions to guide future research topics include:

- If the PFAA inhibition experiments described in Chapter 3 were repeated using the improved experimental design recommended in Chapter 5, would statistically significant differences be observed between the other Monod kinetic parameters? The improved experimental design results in parameter interval estimates with smaller relative margins of error. If significant differences between parameters other than the maximum substrate utilization rates were apparent, then potential mechanisms of MRD inhibition by PFAAs could be inferred.
- Do other more advanced MCMC algorithms determine parameter interval estimates more effectively or efficiently than the Metropolis algorithm? Hamiltonian Monte Carlo (HMC, used in Chapter 5 to select realistic sets of parameter values) is worth trying, but it may be significantly slower than Metropolis if gradients of the likelihood have to be approximated numerically. If the ordinary differential equation (ODE) solver used is compatible with automatic differentiation, then that would preclude the need to approximate the gradient numerically and potentially make HMC a viable option. The TensorFlow Probability (TFP) library maintained by Google has ODE solvers with automatic differentiation capabilities, but I found that efficiency gains are only realized if thousands of ODE systems are solved in parallel. If only a few ODE systems are being solved at once (e.g., when fitting the MRD model with four HMC chains) then using a conventional ODE solver (e.g., SciPy) was much faster (as well as less prone to poorly documented numerical errors).
- Could uncertainty quantification (UQ) methods be utilized to explore the cost surface more effectively and efficiently than the methodology recommended in Chapter 5? For example, conformal prediction is a UQ technique used to assign confidence levels to

output from black box prediction models like neural networks.²³⁹ Could regression analysis of numerical model fitting experiments (e.g., Monte Carlo Experiment 2 from Chapter 5) be combined with conformal prediction to determine how marginal confidence intervals returned by regression analysis should be adjusted so that coverage matches the nominal confidence level? Regression analysis coverage values in Chapter 5 were all below the nominal confidence level of 90 percent, so conformal prediction would suggest eight inflation factors by which the eight marginal confidence intervals returned by regression analysis should be enlarged so that there is a 90 percent probability that each interval contains the true parameter value.

- Could the recommended experimental design described in Chapter 5 be amended with additional experiments that do not greatly increase the cost or labor but greatly decrease the uncertainty of estimated parameters? Possible additions include: (1) an experiment (i.e., microcosm or batch reactor) initialized with a mix of CAH compounds so that competitive inhibition effects can be better estimated, (2) individual experiments initialized with each of the daughter CAH compounds so that uncertainty does not propagate down the MRD chain, (3) an experiment amended with a bacteriostatic agent so that the dechlorinating bacteria population does not grow, allowing non-yield parameters to be estimated more accurately, and (4) experiments initialized with varying amounts of bioaugmentation inoculum.
- How can qPCR measurements of reductive dehalogenase (RDase) genes be leveraged to reduce parameter estimation uncertainty? RDases are a membrane-bound class of enzymes that mediate MRD reactions.²¹ As described in Chapter 3, different *Dhc* strains contain different subsets of the RDase genes, and these different *Dhc* strains complete

different steps of the MRD chain metabolically (i.e., utilizing the resulting energy to fuel population growth), co-metabolically (i.e., without population growth), or not at all.

RDase gene abundance data could be used to fit a version of the MRD model that separates the *Dhc* population into multiple strains with unique Monod kinetic parameters, but fitting a model with more fitting parameters generally has the effect of increasing estimation uncertainty (i.e., increasing estimation interval widths) for all parameters.²⁴⁰

Depending on the experimental design and bioaugmentation inoculum used, a Wilks test (see Chapter 5) could be posed to see if the better model fit achieved by a strain-specific MRD model justifies its use over a model where all *Dhc* are lumped into one population. If the nested model structure required by a Wilks test is not possible to construct, then a more general model discrimination metric (e.g., the Akaike information criterion²⁴¹, the Bayesian information criterion²⁴²) could be used.

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