MODELLING TRICHLOROETHENE METABOLIC REDUCTIVE DECHLORINATION BY A MIXED ANAEROBIC CULTURE AT NON-LIMITING ELECTRON DONOR CONCENTRATIONS

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EXTENDED ABSTRACT

Monod-type kinetics are the most prevalent mathematical expressions for the description of chloroethene biodegradation. Despite the small differences in the mathematical formulations employed in the literature, kinetic parameters that have been estimated for Monod-type models vary by two to three orders of magnitude. Therefore, borrowing kinetic parameters from the literature is not a viable option. Consequently, a simple model using Monod-type kinetics was developed in order to describe trichloroethene (TCE) metabolic transformation to ethene by a mixed anaerobic culture under non-limiting electron donor conditions. The main goal of the present work is to estimate the kinetic parameters utilized in the aforementioned model, determine which of the parameters are critical and finally evaluate the uncertainty of the model's estimates. To this end, three batch experiments were conducted (in duplicates) utilizing either butyrate alone or hydrogen and butyrate as electron donors. In any case, the quantity of the electron donor added to the anaerobic culture was at least 2.4 times greater than the electron acceptor demand for dechlorination, on an electron equivalent basis. Thus, chloroethenes were the only limiting factor that was included in the model. Each dechlorination step was assumed to be metabolic. Two variations of this model were also developed investigating the possibility of (1) the presence of two dechlorinating species and (2) competitive inhibition between chloroethenes. For the kinetic parameter estimation, a stochastic global optimization routine was used. This routine creates multiple pseudo-random starting points in the constrained parameter space. Then, based on a sequential quadratic programming algorithm, it searches for the point that minimizes the sum of the square of the errors and satisfies the constraints. The parameter constraints used herein are a result of a comprehensive literature review on metabolic reductive dechlorination modelled with Monod-type kinetics.

Parameters estimated from the experiments with hydrogen and butyrate as electron donors were able to simulate satisfactorily the experiments where butyrate served as electron donor exclusively. Moreover, the sensitivity analysis performed indicated that the microorganism-related parameters (i.e. maximum specific growth rate and yield) were the most critical, whereas the substrate-related parameters (i.e. half-velocity and inhibition coefficients) were not. Finally, model assumptions regarding the utilization of two dechlorinating populations and inhibition kinetics between DCE and VC were found to be justified, based on the goodness of fit to the experimental data.

Keywords: Trichloroethene, Reductive dechlorination, Monod kinetics, Inhibition, Optimization

1. INTRODUCTION

Due to the wide use of trichloroethene (TCE) as solvent or decreasing agent, TCE and its daughter products [i.e. dichlorinated ethenes (DCEs) and vinyl chloride (VC)] are detected frequently in groundwater (Bradley, 2003). For TCE-contaminated sites,
microbial reductive dechlorination (MRD) can be a viable remediation strategy. The MRD process has been studied extensively in the laboratory and in some cases described with kinetic models, usually Monod-type (Chambon et al., 2013). Despite the small differences in the mathematical formulations of the Monod-type models, the estimated model parameters reported in the literature vary by two to three orders of magnitude. These variations may result from differences in the experimental conditions, the limited quantitative microbial data available (Chambon et al., 2013), or the parameter identifiability problems that are inherent in Monod kinetics (Liu & Zachara, 2001). Hence, estimates of MRD performance based on parameter values from the literature can be highly uncertain.

This paper evaluates three alternative models, through comparisons with the results of batch experiments using an anaerobic mixed dechlorinating microbial culture, and quantifies sources of uncertainty. The starting point is a simple reference model utilizing Monod-type kinetics, which is compared to two variations: one involving two dechlorinating populations and another considering in addition competitive inhibition between TCE products. Accounting for microbial processes was simplified by providing adequate electron donor to the anaerobic culture. Regardless of the electron donor utilized (butyrate or a combination of hydrogen and butyrate), donor quantity added was at least 2.4 times greater than the electron acceptor demand on an electron equivalent basis. As a result, the model needed to account for only one limiting factor, i.e. the chloroethenes.

The work performed involved three steps. We first determined the kinetic parameters describing the hydrogen-fed experiments with a stochastic global optimization algorithm. Then we simulated the butyrate-fed experiments with these same parameters. Finally, we calculated the sensitivity of chloroethene concentrations with respect to the model parameters and evaluated the uncertainty of our estimations.

2. BATCH EXPERIMENTS
Details for the operating conditions of the parent culture, the composition of the growth medium and the analytical methods have been described elsewhere (Panagiotakis et al., 2008). Three batch experiments were conducted in duplicates, which contained 100 ml of liquid at a 6:10 headspace/liquid ratio. TCE and electron donor were added as shown in Table 1. Samples were taken periodically for the analysis of chloroethenes, ethene, methane (the results of which are reported herein as nominal concentrations) and volatile fatty acids. At the end of the experiments, volatile suspended solids and protein concentration were determined for biomass estimation.

Table 1. Electron acceptor to electron donor ratio in the batch experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Electron acceptor TCE</th>
<th>Electron donor Butyrate</th>
<th>Hydrogen</th>
<th>Minimum surplus$^{(1)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen-fed</td>
<td>500 3000</td>
<td>300 1200</td>
<td>3000</td>
<td>6000 2.4</td>
</tr>
<tr>
<td>Butyrate-fed</td>
<td>500 3000</td>
<td>3000 12000</td>
<td>-</td>
<td>- 4.0</td>
</tr>
<tr>
<td></td>
<td>500 3000</td>
<td>9100 36400</td>
<td>-</td>
<td>- 12.1</td>
</tr>
</tbody>
</table>

$^{(1)}$ minimum hydrogen available in µeq/l / electron acceptor demand for MRD in µeq/l, $^{(2)}$ butyrate is assumed to yield hydrogen and acetate according to the reaction: $\text{But}^- + 2\text{H}_2\text{O} \rightarrow 2\text{Ac}^- + 2\text{H}_2 + \text{H}^+$

3. MODEL DEVELOPMENT

3.1 Microbial reductive dechlorination kinetics
Kinetic equations for chloroethene consumption are based on a simple Monod-type model. The general form for the consumption rate of the $i$th chloroethene is the following:
where \( \mu_{\text{max},j} \) is the maximum specific growth rate (d\(^{-1}\)) of microorganism \( j \), \( Y_j \) is the yield coefficient (mg VSS/\mu mol substrate), \( X_j \) is the biomass concentration (mg VSS/l), \( S_i \) is the substrate concentration (\mu M) and \( K_{si} \) (\mu M) is the half-velocity coefficient.

In sequential dechlorination, the consumption of the \( (j) \)th chloroethene is simultaneously the production of the less chlorinated \( (i-1) \)th product. Thus, the differential equation that describes the change in the concentration of chloroethenes is the following:

\[
\frac{dS_i}{dt} = -r_i + r_{i-1}
\]  
(2)

Microbial growth supported by reductive dechlorination is simulated as follows:

\[
\frac{dX_j}{dt} = \sum_i (Y_j \cdot r_i) - (b_j \cdot X_j)
\]  
(3)

where \( b_j \) is the decay coefficient (d\(^{-1}\)).

In Eq. (1) to (3), \( \mu_{\text{max},j} \), \( Y_j \) and \( b_j \) are considered microorganism-related parameters, not associated with the substrate consumed. For \( \mu_{\text{max},j} \) and \( b_j \) this is a reasonable assumption. If these parameters differ from reaction to reaction, the number of parameters to be estimated increases significantly, leading to an overdetermined problem. For \( Y_j \) this assumption is typically inconsistent to the thermodynamic aspect of the problem. Theoretical yields calculated for each chloroethene (Duhamel & Edwards, 2007) demonstrate that \( Y_j \) should not be considered constant. However, the differences are minor. Hence, it would be difficult to estimate yields from experimental data. Consequently, the observed differences in the removal rates with different substrates will affect only half-velocity coefficients in the kinetic equations described above. As already mentioned, electron donor limitations were not taken into account, because electron donors were supplied in excess. In addition, experimental results suggest that methanogenic activity was not able to create limiting conditions (data not shown).

3.1.1 Reference model
The reference model comprises Eq. (1) to (3), which are developed for all chloroethenes and ethene. The main assumption of the reference model is the existence of a single dechlorinating species, which gains energy from the consumption of TCE, DCE and VC.

3.1.2 Model variation 1
The first variation of the reference model aimed to account for the observed differences in consumption rate between TCE and its daughter products, by considering two dechlorinating species that gain energy from TCE consumption. The same mathematical formulation is used, where \( j=1, 2 \) for \( i=\text{TCE} \) in Eq. (1) and (3).

3.1.3 Model variation 2
The second variation of the reference model was developed in order to investigate the possibility of competitive inhibition between DCE and VC, i.e. the preferential degradation of DCE when VC is also available. Competitive inhibition between chloroethenes has already been accounted for in the literature (e.g. Cupples et al., 2004). In our case, Eq. (1) for VC consumption rate is replaced by the following expression:

\[
r_{\text{VC}} = \frac{\mu_{\text{max},j} \cdot X_j \cdot S_{\text{VC}}}{Y_j \cdot K_{x,\text{VC}} + S_{\text{VC}}}
\]  
(4)

where \( K_{inh} \) (\mu M) is the inhibition coefficient. Finally, it is also assumed that there are two dechlorinating species present, as described in Section 3.1.2.
3.2 Model implementation

Every model consists of an initial value problem. Initial values concerning chloroethene concentrations are directly measured at the beginning of each batch experiment. However, specific biomass concentrations for the dechlorinating species cannot be measured. In order to solve the system of differential equations, initial biomass concentrations were estimated from (a) the performance data of the parent culture at steady state (i.e. dechlorination rates on a weekly basis), (b) the assumed values of $Y_j$, $b_j$ and (c) the overall biomass concentration of the parent culture ($X_{w0} = 26$ mg VSS/l). Because yields and decay coefficients were unknown, they were chosen from the literature. Table 2 gives the range of reported $Y_j$ and $b_j$ values in the literature, and Table 3 lists choices made herein, including the estimated biomass fractions.

Table 2. Parameter space constraints based on the values reported from literature

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield, $Y_j$ (mg VSS/μmol Cl⁻)</td>
<td>$1.76 \times 10^{-4}$</td>
<td>$8.96 \times 10^{-3}$</td>
</tr>
<tr>
<td>Growth rate, $\mu_{max}$ (d⁻¹)</td>
<td>$0.01$</td>
<td>$3.42$</td>
</tr>
<tr>
<td>Decay coefficient, $b_j$ (d⁻¹)</td>
<td>$0.024$</td>
<td>$0.050$</td>
</tr>
<tr>
<td>Half-velocity coefficient, $K_{inh}$ (μM)</td>
<td>$0.05$</td>
<td></td>
</tr>
<tr>
<td>Inhibition coefficient, $K_{oe}$ (μM)</td>
<td>$602.00$</td>
<td></td>
</tr>
</tbody>
</table>

(a) Original values converted to mg VSS assuming 1 cell = $1.6 \times 10^{-11}$ mg VSS (Cupples et al., 2003), (b) Holmes et al. (2006), (c) Duhamel et al. (2004), (d) Sung et al. (2003), (e) Huang & Becker (2009), (f) Yu et al. (2005), (g) Friis et al. (2005), (h) Fennell & Gossett (1998), (i) Cupples et al. (2003), (j) Yu & Semprini (2004)

Table 3. Model input and estimated initial biomass concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield, $Y_j$ (mg VSS/μmol Cl⁻)</td>
<td>$4.96 \times 10^{-4}$</td>
</tr>
<tr>
<td>Decay coefficient, $b_j$ (d⁻¹)</td>
<td>$0.024$</td>
</tr>
<tr>
<td>Initial biomass, $X_i$ (mg VSS/l)</td>
<td>$15.72$</td>
</tr>
</tbody>
</table>


The remaining parameters were estimated from the solution of the inverse problem. The bibliographic range of values shown in Table 2 defined the feasible area in the parameter space. Next, we created a Matlab code that utilizes a stochastic global optimization algorithm. This algorithm generates 500 convergence points of a local optimization routine (i.e. a sequential quadratic programming algorithm) from pseudo-randomly generated starting points from the feasible area. The forward problem is solved numerically in Matlab using a numerical differentiation formula for stiff problems.

4. RESULTS

4.1. Model selection based on the hydrogen-fed experiments

In both hydrogen-fed experiments the reference model failed to describe MRD adequately (data for one experiment shown in Figure 1). The different removal rates of TCE and VC were the probable cause of the poor simulation: the optimization routine resulted in a sum of the squared errors approximately equal to $SSE = 7 \times 10^5 \mu M^2$. 

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Figure 1. Reductive dechlorination kinetics for the hydrogen-fed experiments. The lines represent the best fit achieved with the reference model.

Model variation 1 performed better; it described satisfactorily the overall reaction, as well as each step separately (Figure 2). The calculated curves fitted well the observed concentration values, with a sum of squared errors approximately equal to SSE = 1 x 10^5 μM^2. However, in both duplicates the curve-fitting process miscalculated (a) VC peak concentration and (b) VC elimination time.

Figure 2. Reductive dechlorination kinetics for the hydrogen-fed experiments. The lines represent the best fit accomplished with model variation 1.

The aforementioned problems were eliminated with model variation 2, which accounted for inhibition between DCE and VC (Figure 3). As a matter of fact, all MRD steps were simulated successfully. What is more, the lowest sum of squared errors was achieved: SSE = 3 x 10^4 μM^2. As a result, model variation 2 was used to simulate the four experiments where butyrate was utilized as the only electron donor. The optimized parameters for model variation 2 (average values from fitting the data of each duplicate experiment) are shown in Table 4.

Figure 3. Reductive dechlorination kinetics for the hydrogen-fed experiments. The lines represent the best fit accomplished with model variation 2.
Table 4. Maximum specific growth rates ($\mu_{\text{max},j}$), half-velocity coefficients ($K_{s,i}$) and inhibition coefficients ($K_{\text{inh}}$) obtained from the duplicate hydrogen-fed experiments and the model variation 2

<table>
<thead>
<tr>
<th></th>
<th>TCE-to-ETH dechlorinator</th>
<th>TCE-to-DCE dechlorinator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_{\text{max},j}$ (d$^{-1}$)</td>
<td>$K_{s,i}$ (µM)</td>
</tr>
<tr>
<td>Average</td>
<td>0.21</td>
<td>133.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.04</td>
<td>16.0</td>
</tr>
</tbody>
</table>

4.2. Simulating butyrate-fed experiments

Based on the average values of the model parameters presented in Table 4, we simulated the butyrate-fed experiments described in Section 2. The result of a simulation at a 4-fold electron donor surplus is depicted in Figure 4. Both duplicates of the two butyrate-fed experiments had similar performance regardless of the differences in electron donor surplus. Despite the fact that the model is not accounting for butyrate fermentation, simulation curves were drawn satisfactorily close to the observations, with an average sum of squared errors approximately equal to $\text{SSE} = 1 \times 10^5$ µM$^2$. In other words, the amount of butyrate added was proven sufficient to support dechlorination throughout the duration of the experiments, without any lag time due to butyrate fermentation.

Figure 4. Reductive dechlorination kinetics for the butyrate-fed experiments using parameters determined from hydrogen-fed experiments.

4.3 Local sensitivity analysis

The importance of parameters in complex models is not a priori obvious and may be counter-intuitive. Consequently, sensitivity analysis is a useful tool in the study of the dependence of systems on their respective parameters. In order to compute sensitivity, the local partial functional derivatives of the chloroethene concentrations should be calculated with respect to the model parameters.

A simple way to estimate local sensitivities is by recalculating the response of the model, using parameter values that deviate by small amounts from their nominal values. The sensitivities are then calculated using a finite difference approach. This method is conceptually simple and does not require any additional model development. More specifically, we used a uniform approach altering all nominal values by 0.1% in both directions (both decreasing and increasing the nominal concentration values). Finally, we calculated the time integral of the sensitivity coefficients for a six-day long simulation. From the time integrals calculated (Table 5), it is evident that chloroethene concentrations are sensitive to microorganism-related parameters (i.e. $Y_j$, $\mu_{\text{max},j}$ and $b_j$) and not to substrate-related parameters (i.e. $K_{s,i}$ and $K_{\text{inh}}$). In other words, a small perturbation in $Y_j$, $\mu_{\text{max},j}$ or $b_j$ would cause noticeable changes in chloroethene concentrations. Moreover, for these parameters, the VC consumption step is found to be the most critical, as indicated by the maximum values of the time integrals in Table 5.
Table 5. Time integrals of the concentration sensitivities with respect to model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TCE</th>
<th>DCE</th>
<th>VC</th>
<th>ETH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{1}^{[a]}$</td>
<td>2.43 x 10^3</td>
<td>8.87 x 10^4</td>
<td>2.84 x 10^5</td>
<td>2.64 x 10^5</td>
</tr>
<tr>
<td>$Y_{2}^{[b]}$</td>
<td>2.13 x 10^4</td>
<td>2.34 x 10^4</td>
<td>1.03 x 10^4</td>
<td>3.56 x 10^3</td>
</tr>
<tr>
<td>$\mu_{\text{max},1}^{[a]}$</td>
<td>5.93 x 10^1</td>
<td>2.39 x 10^3</td>
<td>8.32 x 10^3</td>
<td>7.84 x 10^3</td>
</tr>
<tr>
<td>$\mu_{\text{max},2}^{[b]}$</td>
<td>2.46 x 10^1</td>
<td>2.67 x 10^1</td>
<td>1.20 x 10^1</td>
<td>0.44 x 10^1</td>
</tr>
<tr>
<td>$b_{ij}$</td>
<td>0.91 x 10^1</td>
<td>3.64 x 10^2</td>
<td>2.58 x 10^3</td>
<td>2.61 x 10^3</td>
</tr>
<tr>
<td>$K_{s,1}^{[c]}$</td>
<td>3.12 x 10^2</td>
<td>3.46 x 10^1</td>
<td>1.03 x 10^1</td>
<td>9.48 x 10^1</td>
</tr>
<tr>
<td>$K_{\text{inh}}$</td>
<td>4.35 x 10^{-5}</td>
<td>7.30 x 10^{-2}</td>
<td>0.90 x 10^1</td>
<td>0.91 x 10^1</td>
</tr>
</tbody>
</table>

(a) TCE-to-ETH dechlorinator, (b) TCE-to-DCE dechlorinator, (c) average values for TCE, DCE and VC

Since yield, decay coefficient and the TCE fraction consumed by each dechlorinating species were obtained directly from the literature (Table 3), they dictated the distribution of biomass fractions. In order to evaluate uncertainty of parameter estimates resulting from these choices, 200 recalculations were conducted by randomly picking different values for yield and decay coefficient from uniform distributions bounded by the values shown in Table 2. TCE fractions consumed by the dechlorinating species were distributed between 50% and 90%. Consequently, each calculation was performed with different initial biomass concentrations. The optimization routine described in Section 3.2 was again performed and gave the results shown in Table 6.

Table 6. Maximum specific growth rates ($\mu_{\text{max},j}$), half-velocity coefficients ($K_{s,j}$) and inhibition coefficients ($K_{\text{inh}}$) based on 200 recalculations with random initial conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TCE-to-ETH dechlorinator</th>
<th>TCE-to-DCE dechlorinator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_{\text{max},j}$</td>
<td>$K_{s,j}$ (µM)</td>
</tr>
<tr>
<td></td>
<td>(d$^{-1}$) TCE DCE VC</td>
<td>(µM)</td>
</tr>
<tr>
<td>Average</td>
<td>0.26</td>
<td>41.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.07</td>
<td>95.3</td>
</tr>
</tbody>
</table>

Tables 4 and 6 indicate that the half-velocity coefficients regarding TCE and DCE may be unreliable, probably due to the small duration of the respective dechlorination steps. On the contrary, maximum specific growth rates, the VC half-velocity coefficient and the inhibition coefficient are estimated with minimal uncertainty.

5. CONCLUSIONS

Experiments with high electron donor surpluses were successfully simulated with a simple Monod-type model, regardless of the electron donor type that was utilized. The best fit was obtained when using two dechlorinating species and accounting for DCE inhibiting VC. From the optimized parameters, maximum specific growth rates, yield and decay coefficients were critical for the overall reaction. Finally, the uncertainty resulting from the lack of culture-specific microbiological data was not coupled with parameter identifiability issues for the majority of the parameters employed in the model, with the exception of the half-velocities for TCE and DCE consumption.

REFERENCES


