Bayesian Estimation of Toluene and Trichloroethylene Biodegradation Kinetic Parameters

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Abstract
Accurate estimation of bioreaction parameters (e.g., the substrate reaction rate constant and the substrate half-saturation parameter in Monod or Monod-derived equations) is critical for successful modeling, reactor design, and scale-up in bioremediation. Conventional maximum likelihood estimation methods are not well suited to estimation of parameters associated with complex nonlinear biological reactions and small-scale experimental data. This paper demonstrates that Bayesian estimation, a standard approach for parameter estimation for physiologically based pharmacokinetic models, is viable for estimating model parameters for such dynamic biological systems. This approach is illustrated using reaction kinetic data from replicated batch experiments for toluene and trichloroethylene (TCE) biodegradation by the microorganism *Pseudomonas putida* F1. This paper evaluates the prediction capabilities of Bayesian estimation by comparing predicted and observed data and reports on goodness-of-fit statistics. The results demonstrate that Bayesian estimation methods can be particularly useful for bioreaction kinetic determination in the presence of small data.

Key Words: Bayesian statistics, toluene and trichloroethylene cometabolism, kinetics parameter estimation

1. Introduction

Trichloroethylene (TCE) is a nonflammable volatile organic compound (VOC) widely used as a cleansing agent or solvent for metal degreasing and solvent extraction in various industries and in household products, such as typewriter correction fluid, paint removers, and spot removers (Agency for Toxic Substances and Disease Registry [ATSDR], 2018b). Careless disposal, spillage, and leakage have made TCE among the most common pollutants found in U.S. contaminated groundwater (Moran, Zogorski, & Squillance, 2007), and it has been detected in at least 1,045 of the 1,699 National Priorities List sites identified by the U.S. Environmental Protection Agency (ATSDR, 2016). TCE's toxicity and possible carcinogenic property (Eder, 1991; Scott & Jinot, 2011) cause great concern when it is found in contaminated groundwater. In addition, toluene, which is used as an industrial solvent (ATSDR, 2018a), is one of the most common pollutants found in groundwater and domestic water supplies in some areas (Hamid, Amin, Alazba, & Manzoor, 2014). TCE together with toluene and other compounds, such as benzene and xylenes, are likely found together as combined pollutants at contaminated industrial sites.

Many studies have been conducted to remove TCE through biotransformation by using various microorganisms via reductive or oxidized dechlorination (Pant & Pant, 2010). Because TCE cannot be used as a carbon source for microorganisms to grow, it can be transformed only through cometabolism under aerobic conditions in which nonspecific oxygenases of microorganisms catalyze the oxidation of TCE in the presence of a primary substrate. These microorganisms include methane-oxidizing bacteria, toluene oxidizers, and phenol oxidizers. For example, when toluene and TCE present together in an environment, toluene can be used as a primary substrate for microorganisms to grow and produce nonspecific oxygenases to degrade TCE into nontoxic compounds. The advantage of using coexisting pollutants as a growth substrate for TCE cometabolism is that the co-occurring pollutants in the environment can be removed simultaneously.

TCE cometabolism kinetics is important for predicting the concentrations of contaminants and modeling or designing bioreactors for TCE treatment. Kinetic parameters can be obtained either directly from the literature or by reversing the engine to solve the problem via model fitting to the experimental data. However, parameters reported in the literature may vary substantially due to different estimation methods or experimental conditions (Alvarez-Cohen, 1998; Mars, Prins, Wietzes, de Koning, & Janssen, 1998; Mii, Morono, Tanji, Unno, & Hori, 2004; Robertson & Button, 1987; Sun & Wood, 1996), which could result in differences in parameter values even for the same kind of microorganism species. Hence, the second option is sometimes necessary to accommodate local experimental conditions for a customized parameter fitting. In that sense, reliable estimation of biodegradation parameters, such as the substrate reaction rate constant and the substrate saturation parameter in the Monod or Monod-derived equations, is the key step toward successful modeling, prediction, and bioreactor design. However, nonlinearity, the multiple highly correlated parameter spaces involved in differential equations, and the small-scale data collected from experiments present great challenges in parameter estimation. The traditional method for biological parameter estimation usually cannot handle high-dimension parameter spaces. Strategically, one has to first decompose the modeling process by doing one compound at a time (i.e., estimating compound-specific parameters by steps), then based on some approximate assumptions one needs to further simplify the model by converting it to linear equations to estimate the parameters. For example, the initial rate method (Shuler & Kargi, 1992) is a linearization method for estimating parameters from simple biological models. Therefore, the traditional method relies on certain modeling assumptions and needs more experimental data in order to achieve the desired precision. Further, when models are complicated with multiple compounds, it is hard to capture the interactions between the compounds if estimation is done separately by stepwise modeling. In term of statistical methods, on the other hand, conventional maximum likelihood estimation methods are not well suited in complex nonlinear biological reactions with limited data because significant bias may occur when experimental datasets are small; moreover, these methods produce poor uncertainty estimates based on the Cramer–Rao bound (Jang & Gopaluni, 2011).

The Bayesian method, however, has been a commonly used approach in statistical inference and parameter estimation in physiology-based pharmacokinetics (PBPK) models (Bernillon & Bois, 2000; Chiu, Okino, & Evans, 2009; Krauss et al., 2013; Zurlinden & Reisfeld, 2015), where data are often limited and are collected dynamically among human subjects in clinical trials. Bayesian inference computes the posterior probability-given evidence, which is proportional to the prior probability and the likelihood of evidence given the hypothesis normalized by the probability of events (i.e., the marginal probability from

all hypotheses) (Jeffreys, 1973). Under the Bayesian framework, prior knowledge of parameter distributions is updated with the information obtained from the experimental data to form posterior distributions. In other words, the joint probability distribution of the parameters is obtained after conditioning them to the data and prior knowledge. The prior knowledge relating to the parameters can come from the past experimental data or values reported in the literature. The major advantage of the Bayesian approach is that it is easy and flexible to implement, and it integrates prior information into the model considerations. By combining the Bayesian approach with Markov Chain Monte Carlo (MCMC) procedures, Bayesian estimates can be appropriately calculated even for small samples (Dunson, 2001). By applying MCMC algorithms **(**Gilks, Richardson, & Spiegelhalter, 1996; Tierney, 1994) to the Bayesian method, once a model converges, one can iteratively draw a large number of samples based on the joint posterior distribution of the model parameters. Hence, estimates of the posterior distribution of parameters in a model can be easily obtained. Engineering applications for this approach have also been found, such as parameter estimation in wastewater treatment to address uncertainties (Sharifi, Murthy, Takács, & Mossoudieh, 2014; Stewart et al., 2017). Parameter estimation for TCE biodegradation from a mixed culture has been explored and reported elsewhere (Kandris, Antoniou, Pantazidou, & Mamais, 2015). This paper attempts to apply the Bayesian method to estimate TCE and toluene biological parameters for a microorganism culture in a dynamic system.

In this paper, parameter estimation was investigated for toluene and TCE biotransformation by *Pseudomonas putida* F1 under batch experimental conditions using the Bayesian method. *Psedomonas putida* F1 is one of the common bacteria (Wackett & Gibson, 1988) that has the capability to mineralize TCE. It can use toluene as the main carbon source for its growth. In the cometabolism reaction, toluene dioxygenase was induced by toluene, which simultaneously catalyzed the reaction of TCE degradation. A three-step hierarchical modeling strategy was employed with increasing complexity in the parameter estimation process: (1) estimate the Monod parameters for toluene degradation only; (2) estimate the parameters for TCE degradation only; and (3) estimate the parameters for toluene and TCE cometabolism. The first two steps were to verify the parameters obtained from the Bayesian method with those obtained from the traditional method (i.e., the initial rate method). The third step in the parameter estimation serves as a verification step that the individual parameters obtained from the separate steps are reliable and additionally as a way to identify if there is any interaction when two compounds are degraded cometabolically. Hence, three different types of mathematical models were employed in sequence: (1) the Monod model with toluene as the only substrate; (2) the Monod model with TCE as the only substrate and its toxicity; and (3) the Michaelis-Menten model with the co-presence of toluene and TCE and competitive inhibition in the cometabolism. The corresponding data were collected over time from batch experiments with toluene only, TCE only, or mixed toluene and TCE as the primary substrate(s). The Bayesian method was employed to estimate the posterior distributions of these kinetic parameters. To account for uncertainties from the experiment and measurement, MCMC simulations were conducted to achieve an optimal solution. Overall model fittings were assessed by the deviance information criterion (DIC) (Spiegelhalter, Best, Carlin, & van der Linde, 2002). In addition, other standard goodness-of-fit measures, such as mean squared error (MSE) and mean absolute error (MAE), which quantify the discrepancy between "observed" concentrations of toluene and TCE and the models' "predicted" concentrations over time, were calculated to measure the models' accuracy. Finally, the prediction capability of the models with newly fitted parameters was examined by comparing the model prediction values with the different sets of experimental data under the three models.

2. Materials and Methods

2.1 Batch Toluene and/or TCE Degradation Data

Data were collected from series batch experiments in 25-milliliter (mL) Mininert® vials (Yu, 1998). *Pseudomonas putida* F1 cell cultures, medium, and toluene and/or TCE were added into the vials to form a final liquid volume at 5 mL for different initial toluene and/or TCE concentration combinations. The vapor-phase volume and liquid-phase volume ratio was kept at 4:1. Toluene and TCE concentrations were recorded over sample time. In this work, the following three groups of data were used:

Group 1. Toluene-only degradation data: initial toluene concentrations at 10 milligrams per liter (mg/L), 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L with three replicated vials (replicates) for each of the starting concentrations. Data from the 30 mg/L experiments were used for parameter estimation.

Group 2. TCE-only degradation data: initial TCE concentrations at 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L with two replicated vials (replicates) for each of the starting concentrations. Data from the 1 mg/L experiments were used for parameter estimation.

Group 3. Toluene and TCE cometabolism data: initial toluene and TCE concentrations at 10 mg/L, 1 mg/L, respectively; 40 mg/L and 1 mg/L, respectively; and 10 mg/L and 5 mg/L, respectively, with three replicated vials (replicates) for each of the starting concentrations. Data from the 40 mg/L and 1 mg/L experiments were used for parameter estimation.

As noted above, for each data group, one initial concentration experiment's data were used for modeling and parameter estimation, and the rest of the experiment's data with initial concentrations different from that were used for testing the predictive ability for each model.

2.2 Mathematical Models

Mass balance in a closed gas-liquid system for toluene or TCE can be written in the following expressions:

$$V^l \frac{dC_i^l}{dt} + V^g \frac{dC_i^g}{dt} = V^l \left(\frac{dC_i^l}{dt}\right)_{\text{bioreaction}} \quad (1)$$

and

$$V^l C_i = V^l C_i^l + V^g C_i^g, \quad (2)$$

where V^l is the liquid-phase volume, V^g is the gas-phase volume, C_i^l is the liquid-phase concentration, C_i^g is the gas-phase concentration, C_i is the total concentration if the substance were not volatile, and i = 1, 2, which refers to toluene or TCE. Equation (1) says that the total rate of change for toluene or TCE is the sum of the rate change in the liquid phase and gas phase and is equivalent to the toluene or TCE biodegradation rate in the liquid phase. Equation (2) says that the total mass of toluene or TCE is the sum of the mass in the liquid phase and the mass in the gas phase. Under rigorous shaking conditions, fast equilibrium can be achieved between the liquid and gas phases for both toluene and TCE without mass transfer resistance between the gas and liquid phases, so the relationship

between the gas-phase concentration and the liquid-phase concentration can be described by Henry's law as follows:

$$C_i^g = H_i C_i^l. \tag{3}$$

Substituting Equation (3) into Equations (1) and (2) yields the following:

$$A_i \frac{dC_i^l}{dt} = \left(\frac{dC_i^l}{dt}\right)_{\text{bioreaction}} \tag{4}$$

and

$$A_i = 1 + \frac{V^g}{V^l} H_i. \tag{5}$$

Equation (4) says that the total rate of toluene or TCE change can be expressed using the liquid-phase concentration and is equivalent to the biodegradation rate in the liquid phase.

Therefore, mathematical models under batch conditions can be described using the liquid-phase toluene or TCE concentration for the corresponding data groups denoted earlier in Section 2.1. Below, the superscript "l" is omitted for all concentration notations in the equations. The total liquid concentration (assuming all compound molecules were in the liquid phase without evaporation) can be obtained by multiplying the liquid-phase concentration with the physical constant A_i.

Model 1: Only toluene presented in the solution as the substrate; hence, the toluene consumption rate can be described by substituting the Monod equation into Equation (4), yielding the following:

$$-\frac{dC_{TOL}}{dt} = \frac{1}{A_{TOL}} \frac{k_{TOL} X C_{TOL}}{K_{s,TOL} + C_{TOL}} \tag{6}$$

and

$$\frac{dX}{dt} = Y_e \left(-A_{TOL} \frac{dC_{TOL}}{dt}\right) - b_d X, \tag{7}$$

where k_{TOL} is the rate constant of toluene, $K_{s,TOL}$ is the substrate half-saturation constant of toluene, X is the biomass concentration, Y_e is the cell yield coefficient, and b_d is the cell decay rate. Note here that the total change of the liquid phase and the gas phase of the toluene contributes to the growth of the bacteria.

Model 2: TCE degradation was conducted by using resting cells in the absence of toluene. After *Pseudomonas putida* F1 grew under toluene, they produced sufficient toluene dioxygenase that can be used to catalyze TCE biodegradation even without the presence of toluene. These bacteria are the resting cells. Equations similar to Equations (6) and (7) can be used to quantify the TCE reaction rate, with the exception that TCE has a toxicity effect to the cells so that there is a transformation capacity involved in the biomass equation:

$$-\frac{dC_{TCE}}{dt} = \frac{1}{A_{TCE}} \frac{k_{TCE} X C_{TCE}}{K_{m,TCE} + C_{TCE}} \tag{8}$$

and

$$\frac{dX}{dt} = -\frac{1}{T_C} A_{TCE} \left(-\frac{dC_{TCE}}{dt}\right) - b_d X, \tag{9}$$

where k_{TCE} is the rate constant of TCE, $K_{m,TCE}$ is the substrate half-saturation constant of TCE, and T_C is TCE transformation capacity, which is defined as the mass of TCE consumed per unit amount of biomass. The inverse of T_C is the cell mass deactivated per the amount of TCE degraded. Similar to Equation (7), the toxicity caused by TCE is the result of the biodegradation of TCE in the liquid phase.

Model 3: This model involves toluene and TCE cometabolism, where toluene and TCE were introduced in the solution and a general competitive enzyme reaction model (i.e., a modified Michaelis-Menten equation) can be used to describe the reaction rate change for toluene and TCE, as follows:

$$-\frac{dC_{TOL}}{dt} = \frac{1}{A_{TOL}} \frac{k_{TOL} X C_{TOL}}{C_{TOL} + K_{s,TOL}\left(1 + \frac{C_{TCE}}{K_{m,TCE}}\right)}, \tag{10}$$

$$-\frac{dC_{TCE}}{dt} = \frac{1}{A_{TCE}} \frac{k_{TCE} X C_{TCE}}{C_{TCE} + K_{m,TCE}\left(1 + \frac{C_{TOL}}{K_{s,TOL}}\right)}, \text{ and} \tag{11}$$

$$\frac{dX}{dt} = Y_e A_{TOL}\left(-\frac{dC_{TOL}}{dt}\right) - \frac{1}{T_C} A_{TCE}\left(-\frac{dC_{TCE}}{dt}\right) - b_d X. \tag{12}$$

Equations (6), (8), (10), and (11) quantify the biodegradation rates of toluene and TCE. Equations (7), (9), and (12) quantify the cell growth rate in the presence of toluene and/or TCE. The growth equations indicate that toluene supports cell growth and TCE generates the toxicity effect on the cells.

In prediction, one can use the above models to solve the differential equations to get the solution given the initial conditions and parameters. One the other hand, parameter estimation involves finding the values of these parameters given the measured data. Therefore, to solve the reversed problem for parameters k_{TOL}, $K_{s,TOL}$, k_{TCE}, $K_{m,TCE}$, and T_C estimation, substance concentrations were measured at certain time intervals during the experiments. Additionally, the initial cell density X was measured and was in the range of 390 to 430 mg/L. However, cell density was not measured during the experiments. Other parameters in the models (i.e., the toluene yield coefficient Y_e and the cell decay rate b_d) were treated as known parameters. The toluene yield coefficient Y_e was obtained from a separate set of experiments with a value of 0.4872 mg cell/mg toluene. Various values have been reported for the biomass specific decay rate. For example, 0.0082 (1/h) was reported from Mars et al. (1998), which is equivalent to 0.1968 (1/day); 0.06 (1/h) was reported from Sander (2002), which is equivalent to 1.44 (1/day); and 0.39 (1/day) was reported by Johnson, Park, Kukor, and Abriola (2006). Given this diversity reported in the literature, the average of these values was taken, and 0.68 (1/day) was used as the cell decay constant b_d for the model.

2.3 Bayesian Estimation and Computational Software

In Bayesian statistics, parameters are treated as unknown random variables, and inferences are made based on the posterior distributions of the parameters, where a posterior distribution is proportional to the product of the likelihood function and the prior distribution of the parameter (Jang & Gopaluni, 2011). For the single-compound toluene or TCE biodegradation case, the posterior parameter distribution $p(\boldsymbol{\theta}_i|\boldsymbol{y})$ given observed data $\boldsymbol{y}$ can be described as follows:

$$p(\boldsymbol{\theta}_i|\boldsymbol{y}) = \frac{\prod_{j=1}^{N} p\left(\boldsymbol{C}_{i,j}\middle|\boldsymbol{\theta}_i\right)p(\boldsymbol{\theta}_i)}{\int \prod_{j=1}^{N} p\left(\boldsymbol{C}_{i,j}\middle|\boldsymbol{\theta}_i\right)p(\boldsymbol{\theta}_i)\, d\theta_i}, \tag{13}$$

where $\boldsymbol{C}_{i,j} = \left[C_{i,j}(t_{1,j}), C_{i,j}(t_{2,j}), \ldots, C_{i,j}\left(t_{T_j}\right)\right]$, which is a vector of the measured concentrations over time for the replicated experiment *j*; T_j is the end sample time point for replicate experiment *j*; i = 1 or 2 for the toluene- and TCE-only experiment, respectively; and *N* is the total number of replicated experiments. For the toluene-only model, $\boldsymbol{\theta}_i = [k_{TOL}, K_{s,TOL}]$. For the TCE-only model, $\boldsymbol{\theta}_i = [k_{TCE}, K_{m,TCE}, T_C\,]$, $p(\boldsymbol{\theta}_i)$ is the prior distribution of the parameters, and $p\left(\boldsymbol{C}_{i,j}\middle|\boldsymbol{\theta}_i\right)$ refers to the likelihood of observing the data given the values of the parameters $\boldsymbol{\theta}_i$. For the toluene and TCE cometabolism cases, $\boldsymbol{\theta} = [k_{TOL}, K_{s,TOL}, k_{TCE}, K_{m,TCE}, T_C\,]$. The posterior distribution of the parameter vector $\boldsymbol{\theta}$ needs to account for the joint distributions of both compounds:

$$p(\boldsymbol{\theta}|\boldsymbol{y}) = \frac{\prod_{j=1}^{N} p\left([\boldsymbol{C}_{1,j}, \boldsymbol{C}_{2,j}]\middle|\boldsymbol{\theta}\right)p(\boldsymbol{\theta})}{\int \prod_{j=1}^{N} p\left(\left[\boldsymbol{C}_{1,j}, \boldsymbol{C}_{2,j}\right]\middle|\boldsymbol{\theta}\right)p(\theta)\, d\boldsymbol{\theta}}. \tag{14}$$

The $[\boldsymbol{C}_{1,j}, \boldsymbol{C}_{2,j}] = ([C_{1,j}(t_{1,j}), C_{2,j}(t_{1,j})], [\,C_{1,j}(t_{2,j}), C_{2,j}(t_{1,j})], \ldots, [C_{1,j}(t_{T_j}), C_{2,j}(t_{T_j})])$ in Equation (14) are measured concentrations of toluene and TCE over time, respectively.

Computing the posterior distributions of the parameters is not straightforward because estimating the likelihood functions— in the numerators of Equations (13) and (14) above— involves differential equations, and performing integration in the denominators is difficult due to the high-dimensional spaces. In this study, the system of nonlinear problems from Equations (6) to (14) was solved by employing the PROC MCMC procedure in SAS/STATS® 14.1 (SAS Institute, Inc., 2014). This procedure enables users to fit Bayesian models via MCMC simulations. It is a simulation-based procedure and also allows users to call the ODE solver to solve the differential equations. Within this procedure, MCMC simulations generate samples from the desired posterior distributions, then utilize these simulated samples to approximate the parameter distributions. Distribution inputs are summarized below for the PROC MCMC procedure in SAS. For the chemical concentrations in Models 1 and 2, the normalized concentration $\mu_{i,j}(t_{k,j})$ at a certain time point is defined as follows:

$$\mu_{i,j}(t_{k,j}) = \overline{C_{i,j}(t_{k,j})}, \tag{15}$$

where *i* is the index for toluene and TCE, *j* is the index of the individual replicated experiments in the batch experiment, *k* = 1, 2,…, T_j for the sample point, and $C_{i,j}(t_{k,j})$ is the toluene or TCE concentration at time t_k for vial *j*. The $\overline{C_{i,j}(t_{k,j})}$ is the expected concentration at the time point, which can be computed from $\frac{C_{i,j}(t_{k,j})}{Dp_{i,j}}$, where $Dp_{i,j}$ is the reactant depletion in vial *j*. Then the likelihood function for toluene or TCE can be described as follows:

$$C_{i,j}(t_{k,j}) \sim \text{normal}(\mu_{i,j}(t_{k,j}), \sigma^2). \tag{16}$$

Equation (16) indicates that at each time point, the toluene or TCE concentration in a vial follows a normal distribution with a mean $\mu_{i,j}(t_{k,j})$ and a variance σ^2. Here, random errors

in the measured concentraions include the sample draw error, measurement error, and vial variations. To facilitate the computation, kinetic parameters were first applied to the log-transformation, and to account for variability between the vials, a random term was introduced in the compound depeletion rates Dp_{TOL} and Dp_{TCE}. So, each parameter in Models 1 and 2 were modeled as follows (SAS Institute, Inc., 2014):

For Model 1:

$$k_{TOL} = \exp(\beta_{1r}), \tag{17}$$
$$K_{s,TOL} = \exp(\beta_{1s}), \tag{18}$$
$$Dp_{TOL} = \exp(\beta_1 + b_1), \tag{19}$$

For Model 2:

$$k_{TCE} = \exp(\beta_{2r}), \tag{20}$$
$$K_{m,TCE} = \exp(\beta_{2s}), \tag{21}$$
$$T_C = \exp(\beta_{tc}), \text{ and} \tag{22}$$
$$Dp_{TCE} = \exp(\beta_2 + b_2), \tag{23}$$

where the β's are the fixed effects and the b's are the random effects. The random effect terms b_1 and b_2 enter the model through the depletion variable. Here, random variation due to handling or measurement error during the experiment was lumped into one parameter, as shown in Equations (19) and (23).

For Model 3, although toluene and TCE are biodegradated under cometabolic conditions—that is, the bioreaction follows Equations (10) to (12) in Model 3—the measured toluene and TCE concentrations at each time point follow a multivariate normal distribution, as follows:

$$\begin{pmatrix} C_{TOL,j}(t_{k,j}) \\ C_{TCE,j}(t_{k,j}) \end{pmatrix} \sim \text{MVN}\left(\mu = \begin{pmatrix} \mu_{TOL,j}(t_{k,j}) \\ \mu_{TCE,j}(t_{k,j}) \end{pmatrix}, \Sigma = \begin{pmatrix} \sigma_{11} & \sigma_{12} \\ \sigma_{21} & \sigma_{22} \end{pmatrix}\right), \tag{24}$$

where Σ is the variance and covariance matrix. For such cases in Model 3, the parameters to be estimated are all of the parameters indicated in Equations (17) to (23).

Prior distributions of all of the betas in Equations (17) to (23) are assumed to follow a normal distribution with the means and standard deviation obtained from past experimental data. The random effect b's in Equations (19) and (23) follow a normal distribution with a mean 0 and standard deviations similar to the betas. The prior distributions of the variances for the toluene or TCE concentrations in Equation (16) at each time point follow the inverse gamma distributions with a shape parameter 3 and a scale 2. For Model 3, the random effect b turns into a multivariate normal distribution with a mean 0 and a variance and covariance matrix. The prior distribution of the covariance matrix in Equation (24) and the covariance matrix for the random effect b's for Model 3 is assumed to follow the inverse Wishart distribution with 2 degrees of freedom and a symmetric positive definite scale array S, where S is a 2×2 identity matrix.

2.4 Goodness of Fit

The DIC (Spiegelhalter et al., 2002) was used to assess the goodness of fit of the model fittings, where the deviance is twice the difference between the log likelihood of the probability function based on the data and the log likelihood of a probability function, with the normalizing constants based on the model. The DIC is defined as follows:

$$DIC = \overline{D(\boldsymbol{\theta})} + p_D, \tag{25}$$

where $\boldsymbol{\theta}$ is the vector of the parameters, $\overline{D(\boldsymbol{\theta})}$ is the posterior mean of deviance, and p_D is the effective number of parameters.

The $\overline{D(\boldsymbol{\theta})}$ is a Bayesian measure on how well the model fits the data:

$$\overline{D(\boldsymbol{\theta})} = E_{\boldsymbol{\theta}|\boldsymbol{y}}\left[-2\ln\big(f(\boldsymbol{y})\big) - 2\ln p(\boldsymbol{y}|\boldsymbol{\theta})\right], \tag{26}$$

where $p(\boldsymbol{y}|\boldsymbol{\theta})$ is the likelihood function (i.e., the conditional joint probability density function of the data given the unknown parameters; $f(\boldsymbol{y})$ is a function of the data; and p_D is a penalty term for increasing model complexity. The latter is defined as the difference between the posterior mean of the deviance and the deviance of the posterior mean $\overline{\boldsymbol{\theta}}$ of the parameters:

$$p_D = \overline{D(\boldsymbol{\theta})} - D(\overline{\boldsymbol{\theta}}) = E_{\boldsymbol{\theta}|\boldsymbol{y}}[-2\ln p(\boldsymbol{y}|\boldsymbol{\theta})] + 2\ln p(\boldsymbol{y}|\overline{\boldsymbol{\theta}}), \tag{27}$$

where $D(\overline{\boldsymbol{\theta}})$ is the deviance of the posterior mean $\overline{\boldsymbol{\theta}}$**,** which in turn is defined as follows:

$$D(\overline{\boldsymbol{\theta}}) = -2\ln\big(f(\boldsymbol{y})\big) - 2\ln p(\boldsymbol{y}|\overline{\boldsymbol{\theta}}). \tag{28}$$

Besides using the DIC as the overall model-fitting criterion, standard goodness-of-fit statistics (also called standard deviation measures), which quantify the discrepancy between the experimental data and the predicted data from the fitted model at each time point, were calculated to examine the model fitting. They include MSE and MAE:

$$\text{Mean Squared Error: } MSE = \frac{1}{\sum_{j=1}^{N} T_j}\sum_{j=1}^{N}\sum_{k=1}^{T_j}\left(\hat{C}_{i,j}(t_{k,j}) - C_{i,j}(t_{k,j})\right)^2 \tag{29}$$

and

$$\text{Mean Absolute Error: } MAE = \frac{1}{\sum_{j=1}^{N} T_j}\sum_{j=1}^{N}\sum_{k=1}^{T_j}\left|\hat{C}_{i,j}(t_{k,j}) - C_{i,j}(t_{k,j})\right|, \tag{30}$$

where i = 1, 2 for toluene and TCE, respectively; $\hat{C}_{i,j}(t_{k,j})$ is the predicted posterior mean concentration at a specific time for the k-th data point, j-th replicate; $C_{i,j}(t_{k,j})$ is the observed concentration measured at a specific time for the k-th data point, j-th replicate; and $\sum_{j=1}^{N} T_j$ is the total number of data points.

The MSE is the average of the squared deviations between the observed and predicted scores, and it is a standard measure of goodness of fit in modeling approaches. The MAE is the average of the absolute differences by which an observed value differs from the predicted value. These values close to 0 denote better predictions.

3. Results

3.1 Convergence Diagnostics

In the MCMC simulation, 100,000 iterations for each of the three biodegradation models were run for the corresponding experiment group defined in Section 2.1 with initial concentrations of (a) 30 mg/L toluene and (b) 1 mg/L TCE for single-compound models and (c) 40 mg/L toluene and 1 mg/L TCE for the dual-compound model. The thinning rate was set to 10 for all simulations. Prior parameters, including the means and standard

deviations of the parameters in Equations (17) to (24), were adjusted until model convergence. Diagnostic plots, including trace plots, autocorrelation function plots, and kernel density plots, were output to examine model convergence. The trace plot demonstrates the degree of chain mixing, the density plot presents the smoothed histogram of the posterior distribution of the parameter of interest, and the autocorrelation function plot measures serial correlation between lags from the simulation. A convergence criterion was achieved for all chains. An example of these diagnostic plots is displayed in Figure 1 for β_{1r} from Equation (17) for toluene-only biodegradation, where β_{1r} is the log transformation of the reaction rate constant of the toluene biodegradation. The trace plot in Figure 1 shows that the chains were well mixed from the posterior samples, and the autocorrelation plot indicates that the sample draw autocorrelation between the terms of the chain died out quickly. The density plot reveals a nearly standard normal distribution of the posterior estimates of parameter β_{1r}. All of these diagnostic plots provide good evidence of chain convergence. Similar diagnostic plots that show good convergence was achieved for the parameters from Equations (18) to (23) (diagrams not shown).

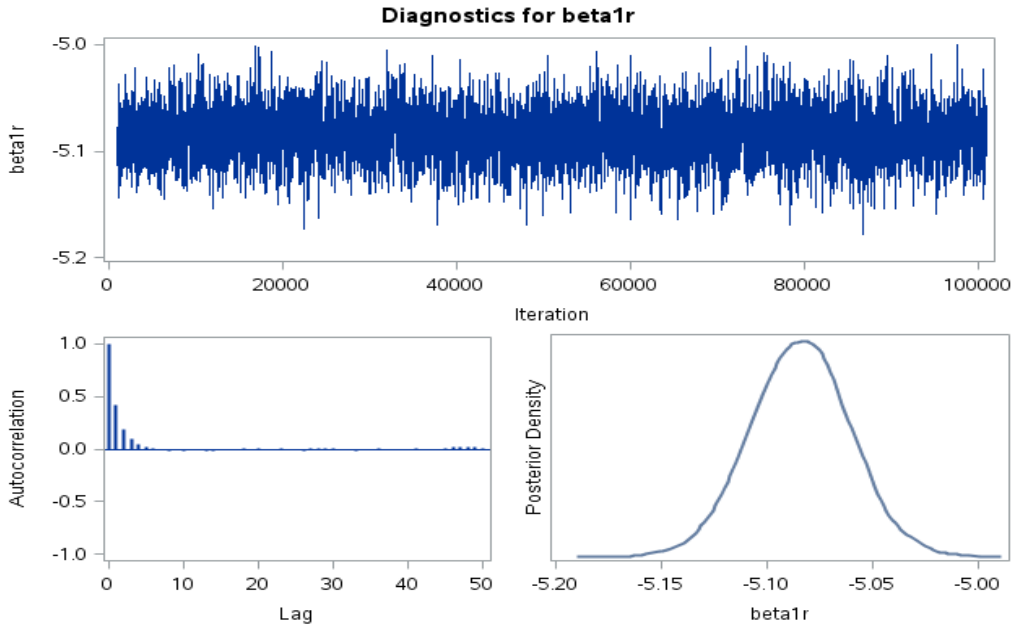

Figure 1: Example diagnostic plots for β_{1r} from Equation (17) for single-compound toluene biodegradation

3.2 Goodness of Fit

As mentioned in Section 2.4, the goodness of fit for the three models was measured by the DIC, MSE, and MAE. The DIC measures were directly read from the SAS PROC MCMC output. The MSE and MAE measures were calculated by using the observed toluene and/or TCE concentrations at each time point and their corresponding model-predicted values, which were the means of the posterior distribution of the toluene and/or TCE concentrations at that specific time from the MCMC simulation. The results of the goodness-of-fit assessment are provided in Table 1.

Table 1: Goodness of Fit of Predicted Means Using the Fitted Model Compared with the Experimental Data

	Toluene				*TCE*			
Model	*DIC*	*MAE*	*MSE*	n[1]	*DIC*	*MAE*	*MSE*	n[1]
1	62.44	1.13	2.22	33	--	--	--	--
2	--	--	--	--	0.01	0.04	0.002	22
3	1.17	0.92	3.21	63	1.17	0.07	0.008	63

[1] n is the total number of data points ($\sum_{j=1}^{N} T_j$) denoted in Equations (29) and (30).

Table 1 shows that except for a relatively large DIC for the toluene-only model, all of the other goodness-of-fit measures were small, specifically for the TCE concentration estimates. The MSE and MAE were all close to zero, either from the TCE-only model or from the toluene and TCE cometabolism model. For all of the measures, the desire was to have values as close to zero as possible; however, with the 62.44 value for the DIC from the toluene-only simulation model, the impact on parameter estimation was small. Figure 2 lays out the model fit with means and 95% credible intervals in comparison with the experimental data. These results show that all three models had good fit to the experimental data using the Bayesian approach.

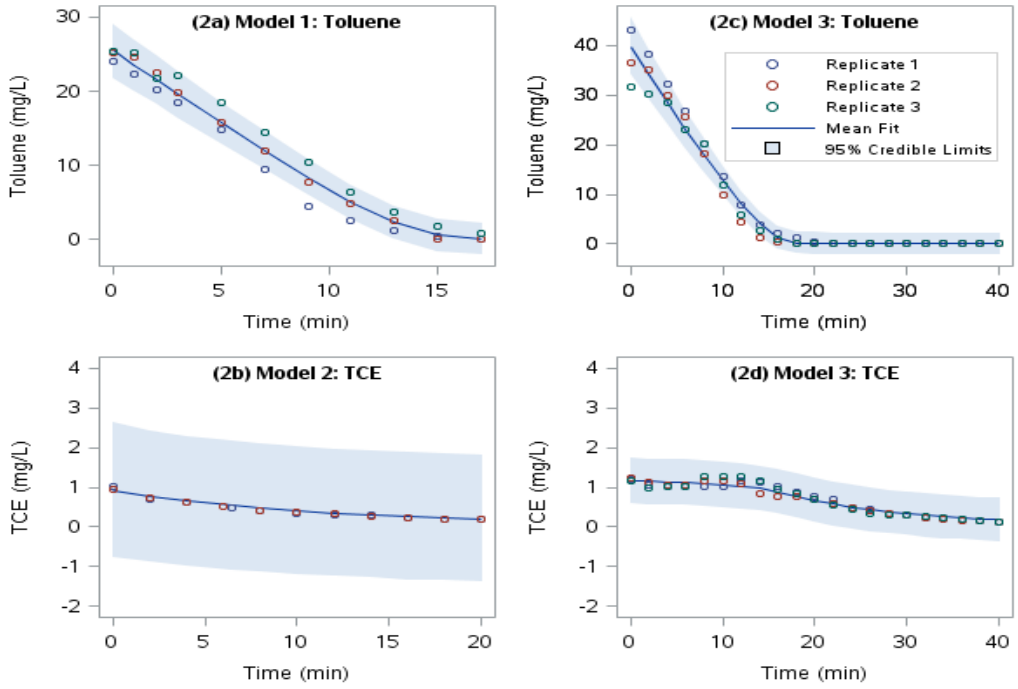

Figure 2: Comparison of model predictions, 95% credible intervals, and experimental data

3.3 Parameters Estimated from the Bayesian Method

The MCMC simulation produced posterior distributions of all betas described in Equations (17) to (23). These distributions were then converted back to the original scales for the rate constants and half-saturation parameters for toluene consumption, TCE degradation, and the TCE transformation capacity (T_C), partially or jointly using these equations depending on the models. Besides the means of the parameters, the 95% credible intervals were also calculated to show the range of likely values of the posterior distributions. The Bayesian estimates of the parameter values under each model scenario are summarized in Table 2.

Table 2: Means and 95% Credible Intervals (C.I.) of Parameters Estimated from the Bayesian Approach and Comparisons with Estimations Obtained from the Traditional Method in a Batch Culture

	Toluene				*TCE*					
	Rate constant k_{TOL} (1/day)		*Substrate half-saturation constant $K_{s,TOL}$ (mg/L)*		*Rate constant k_{TCE} (1/day)*		*Substrate half-saturation constant $K_{m,TCE}$ (mg/L)*		*TCE transformation capacity (T_C)*	
Model	*Est.*	*95% C.I.*	*Est.*	*95% C.I.*	*Est.*	*95% C.I.*	*Est.*	*95% CI*	*Est.*	*95% C.I.*
1 (Tol)	8.92	(8.51, 9.36)	1.11	(1.00, 1.21)	--	--	--	--	--	--
2 (TCE)	--	--	--	--	1.60	(1.33, 1.96)	1.56	(1.28, 1.89)	0.0041	(0.0033, 0.0050)
3 (Tol + TCE)	10.89	(10.47, 11.31)	0.97	(0.82, 1.17)	1.60	(1.36, 1.90)	1.62	(1.36, 1.95)	0.0043	(0.0035, 0.0052)
Traditional (Yu, 1998)	8.44	--	1.11	--	1.58	--	1.52	--	0.0047	--

Table 2 shows that the Bayesian estimates obtained from the single-compound model fittings (Models 1 and 2) using data from the independent experiments were very similar to the estimates using the full-scale Model 3 where TCE and toluene were degraded cometabolically, with the evidence that their 95% credible intervals were highly overlapped. The exception was the toluene rate constant k that the value of 10.89 day^{-1} obtained from the full-scale Model 3 was significantly larger than that obtained from the toluene-only model (i.e., 8.92 day^{-1}). The reason for this noticeable difference is addressed in the discussion section that follows. These results are also supported by the plots of the posterior distributions of the parameters from the simulations (Figure 3).

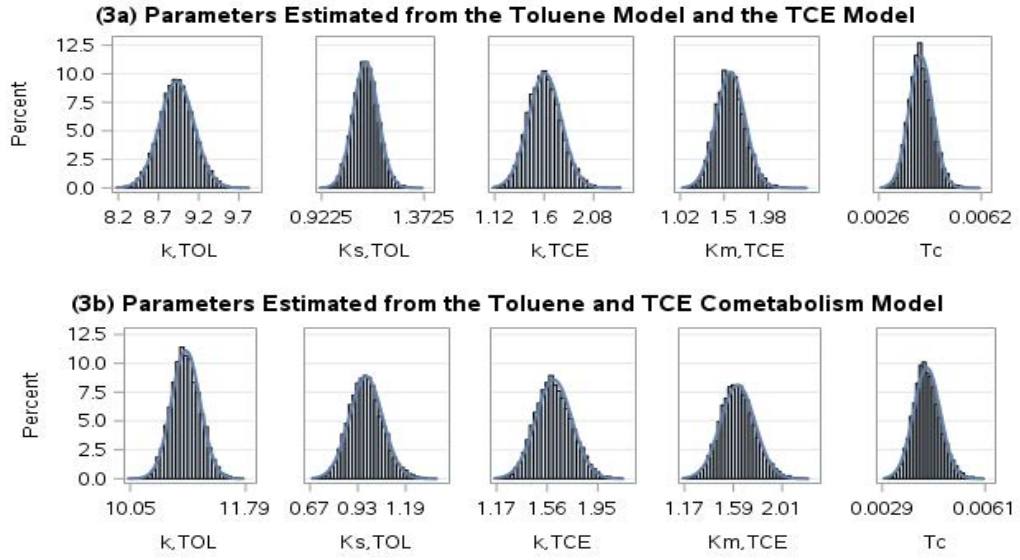

Figure 3: Posterior distributions of model parameter comparisons

As seen in Figure 3, the parameter values from the simulations were consistent with the biological facts that the toluene reaction was faster than that of the TCE. That is, the rate constant of toluene (8.92 day^{-1}) was much higher than that of the TCE (1.6 day^{-1}). Toluene had a higher affinity to the nonspecific oxygenase than TCE, reflecting at the half-saturation parameter that the toluene Ks (1.0 – 1.1 mg/L) was lower than the TCE Km (1.6

mg/L). (Note that the lower the Ks or Km is, the higher affinity it is to the oxidation enzyme.) The TCE transformation capacity (T_C) acquired from both the single-compound TCE model and the dual-compound toluene and TCE cometabolism model was in the similar magnitude of 0.004.

In addition to the parameter values from the model simulation, estimates from the traditional method (Yu, 1998) (i.e., the initial rate method) are also presented in Table 2. The estimates from Bayesian method for the toluene-only or TCE-only models were very close to those obtained from the traditional method. This provides reassurance that the estimates from the Bayesian method are robust and can be used in biological parameter estimation.

Further, the kinetic parameters obtained from this study were compared with values reported in the literature, as can be seen in Table 3. This table shows that each study produces its unique set of kinetic parameters for toluene and TCE biodegradation. The results from the current study were within the ranges of those values, but they were more in line with those reported by Mii et al. (2004), except for the toluene rate constant, where their value was much higher than that obtained from this study.

Table 3: Comparison of Kinetic Parameters with Previous Studies for *Pseudomonas putida* F1

Source	k_{TOL} *(mg tol/mg cell/day)*	$K_{s,TOL}$ *(mg/L)*	k_{TCE} *(mg TCE/mg cell/day)*	$K_{m,TCE}$ *(mg/L)*	T_c *(mg TCE/mg cell)*
Alvarez-Cohen (1998)	--	--	--	--	0.0052
Mars et al. (1998)[b]	15.6	0.14	--	--	--
Mii et al. (2004)[b,c]	38.48	0.48	1.10	2.23	--
Reardon et al. (2000)	20.64	13.8	--	--	--
Robertson and Button (1987)	0.48	0.06	--	--	--
Sun and Wood (1996)	--	--	1.51[a]	0.66	--
Current study	8.92	1.11	1.60	1.56	0.0041

Note: For toluene or TCE rate constant, if the unit is hr^{-1} in the original literature, it has been converted to day^{-1} in this table.

[a] This was measured in the unit of mg/(day mg protein).

[b] Original units were converted for comparison purposes based on toluene's molecular weight at 92.14 g/mol.

[c] Original units were converted for comparison purposes based on TCE's molecular weight at 131.4 g/mol.

3.4 Comparison of Experimental Data with Model Predictions

To verify that Bayesian estimates of biological parameters can be used to predict toluene and TCE biotransformation, these parameter values were plugged into Equations (6) to (12), then these differential equations were solved numerically under various initial concentrations for Models 1 to 3. The numerical solutions of total concentrations—converted by multiplying the liquid concentrations by a factor of A_i from Equation (5)—were then plotted against the experimental data.

Figure 4 compares the model prediction kinetics using the toluene-only and TCE-only bioreaction model parameters (i.e., the first row in Table 2) with experimental data under various starting concentrations: (a) 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L for toluene; and (b) 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L for TCE. The results show that the toluene or TCE biodegradation kinetics using the parameter values estimated by the Bayesian method provided good prediction at different initial concentrations for toluene and low concentrations of TCE. When the initial TCE concentrations increased, the model

predictions tended to have more departures from the experimental data (see the 2 mg/L TCE degradation). This indicates that the TCE toxicity was possibly a function of the TCE concentration itself and that a revised mathematical model—Equations (8) and (9)—may be needed to account for this.

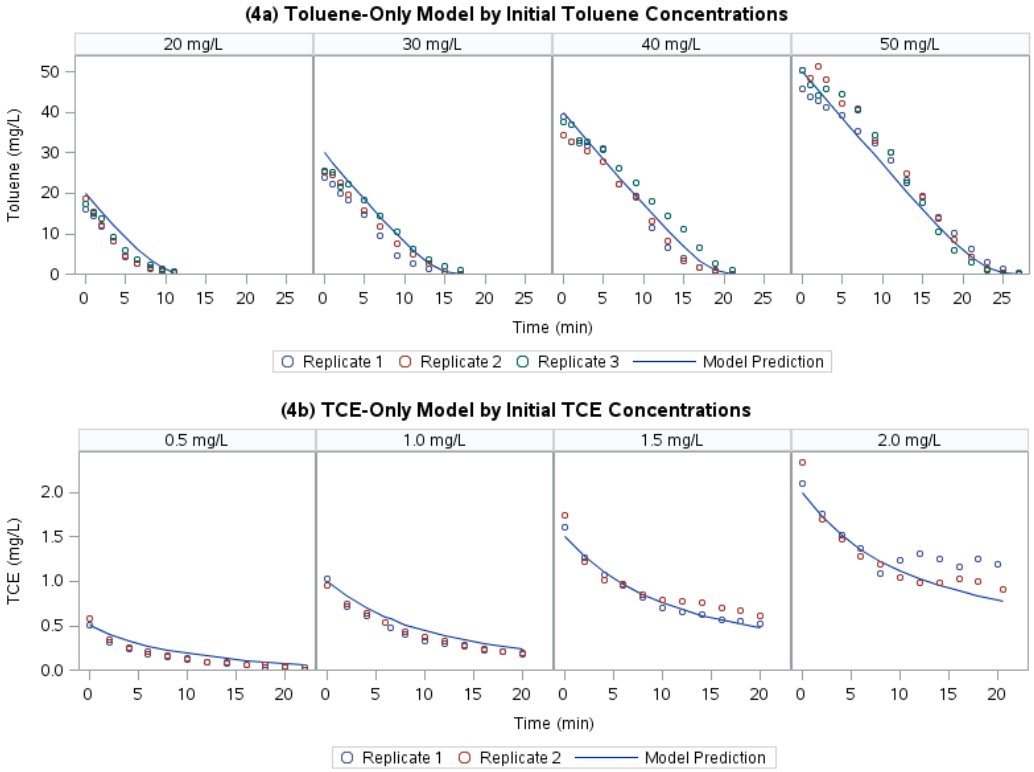

Figure 4: Comparison of model prediction and experimental data by different initial concentrations

Figure 5 shows the predicted kinetics from Equations (10) to (12) using the bioreaction parameters estimated from the Bayesian method (third row in Table 2) along with the experimental data for the toluene and TCE cometabolism. Similar to the single-compound models for toluene or TCE, the dynamic nonlinear differential equations were solved numerically using the fitted parameter values to obtain the predicted toluene and TCE concentrations at different time points. The results were compared with the experimental data graphically for three experiment sets starting at (a) 10 mg/L toluene and 1 mg/L TCE, (b) 10 mg/L toluene and 5mg/L TCE, and (c) 40 mg/L toluene and 1 mg/L TCE.

The results in Figure 5 show that there was good agreement between the model predictions and the experimental data. For the toluene and TCE cometabolism, if the toluene concentration was low, the toluene inhibition to the TCE biodegradation was not so obvious (see the 10 mg/L toluene and 1 mg/L TCE case and the 10 mg/L toluene and 5 mg/L TCE case). That is, the TCE was immediately consumed by the microorganism when compounds were introduced into the reaction vials. However, when the toluene concentration was high, the TCE concentrations stayed similar at the beginning of the reaction and did not become degraded quickly right away (i.e., there was a lag time). This is the effect of the competitive inhibition of toluene in that toluene competes with TCE to take the active site of nonspecific dioxygenase. Once toluene had been depleted, the TCE

concentration started to drop rapidly (see the 40 mg/L toluene and 1 mg/L TCE experiment at time 15 minutes). The TCE toxicity effect was also reflected in the kinetic curve that the rate of changes went down when more TCE had been degraded, as shown by the slope of the TCE degradation curve decreasing toward the end of the experiment. This toxicity effect was more substantial when TCE concentration was high (see the 10 mg/L toluene and 5 mg/L TCE case).

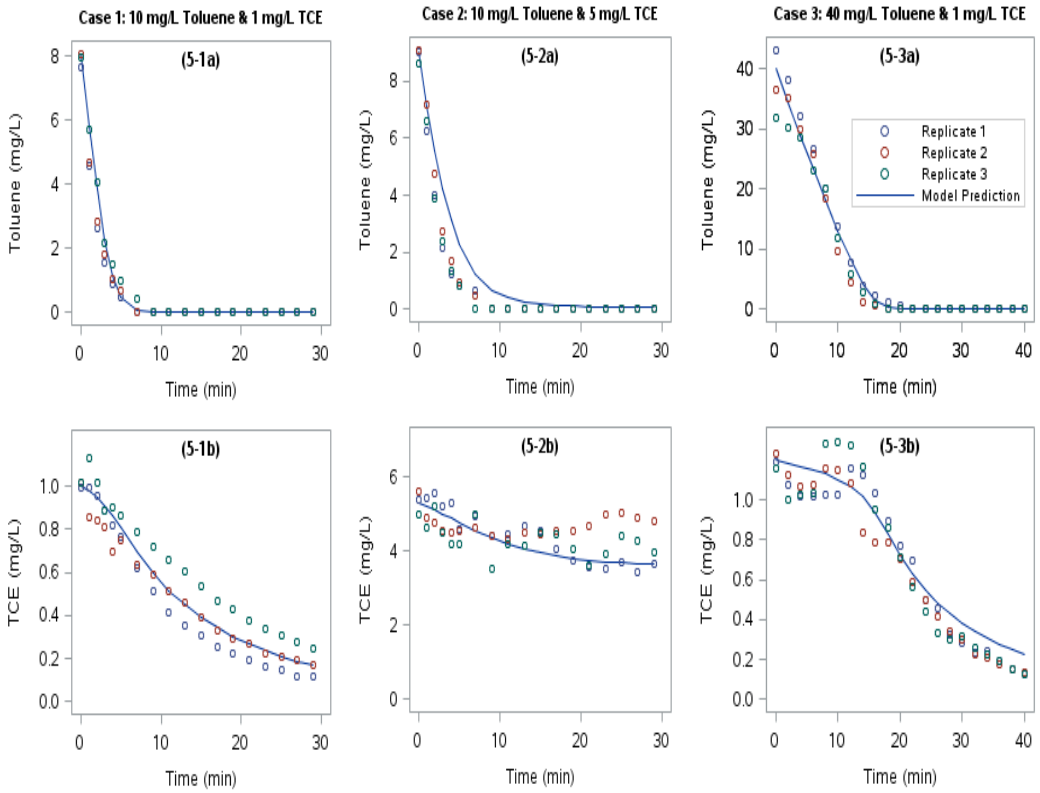

Figure 5: Model prediction and experimental data: toluene and TCE cometabolism

4. Discussion

4.1 General Comments on Parameter Estimation

Parameters in mathematical models play critical roles in modeling, prediction, reactor design, and process implementation. The source of these parameters can come from the literature or model fitting using experimental data. Sometimes, the literature reported different values (Kandris et al., 2015) for the same parameters; at other times, under different experimental conditions, the observed parameters may have different values even when the same microorganism was used (Table 3). This may be due to the fact that the toluene and TCE reaction can be affected by various factors, such as the level of toluene induction, the medium used, the level of oxygen supply, and (consequently) the kinetics. For example, Leahy, Byrne, and Olsen (1996) reported that the growth rate for *Pseudomonas putida* F1 was 10.08 day^{-1} when grown on lactate, which is different from the growth rate when toluene was used as the substrate (e.g., 15.6 day^{-1}, as reported by Mars et al., 1998). In such cases, parameter estimation is required for the local system by solving the reverse problems. When systems are highly nonlinear and not much data are collected, methods other than traditional approaches (such as the initial rate approach and

the maximum likelihood estimation approach) are needed to perform parameter estimation, which rely less on assumptions. The approach described in the current study using the Bayesian estimation method has provided a promising way for conducting parameter estimation under such situations for biological and engineering applications.

In this study, some negative values were observed from the simulation in the posterior distribution of the TCE concentration (Figure 2) that cannot happen in reality. However, the mean fitted values still provided good prediction. In addition, when fewer replicated data were in the simulation, there were more variations, which is reflected in the plot that the 95% credible interval band for the TCE model simulation was wider than that of toluene (Figure 2). The widened 95% credible interval band can be overcome by using more replicates.

4.2 Interaction of Toluene and TCE on Biodegradation

Although Monod-type equations—Equations (6) and (8)—or Monod-derived equations (e.g., the Michaelis-Menten equations)—Equations (10) and (11)—are highly nonlinear and can have nonunique solutions, both Bayesian estimation approaches using either of the single-compound models (Models 1 and 2) or a comprehensive model (Model 3) provided similar posterior estimates for the toluene and TCE kinetics (Table 2). That is, they had good overlapping coverage of 95% creditable intervals, except for the toluene rate constant in which the credible interval from the single substrate model was (8.51, 9.36) and that from the cometabolism model was (10.47, 11.31). Estimates of TCE degradation from the single substrate model were consistent with those from the cometabolism model, which makes sense because in the cometabolism model, the TCE would not start to react until the toluene had been depleted. That is, the TCE was basically degraded as a single substance without much impact from the toluene. For toluene, on the other hand, under the single substrate model, using the traditional method and the single substrate model, estimates for the toluene rate constant had good agreement, with values of 8.44 mg/mg day^{-1} and 8.92 mg/mg day^{-1}, respectively. However, for toluene degradation in the presence of TCE, its reaction rate may have been affected by the TCE, which was therefore reflected in the departure of the toluene rate constant under the cometabolism scheme.

A study by Shingleton, Applegate, Nagel, Bienkowski, and Sayler (1998) showed that the toluene degradation rate by *Pseudomonas putida* TVA8 and *Pseudomonas putida* F1 was a function of the toluene and TCE concentrations. Shingleton et al. (1998) demonstrated that the TCE had the capability to induce the *tod* operon that encodes the toluene dioxygenase. In the presence of both TCE and toluene, dioxygenase activity is superimposed on the toluene inducement, which in turn increases the toluene degradation rate. Although the TCE had its toxicity effect on the cells, its toxicity resulted from the reaction products of the TCE oxidation (Li & Wachett, 1992), not the TCE itself. Its metabolites modified the proteins and reduced the nucleotides in the solution, thus causing a decrease in the growth rate or cell death (Heald & Jenkins, 1994). Under the cometabolism mechanism, the TCE was likely not to be readily degraded until the toluene was depleted due to competitive inhibition. Therefore, before most of the toluene had been consumed, the TCE had not really been oxidized yet, and thus its toxicity was small. In such cases, the increase in dioxygenase enzyme activity surpassed the TCE toxicity, which is why the toluene rate constant was higher in the presence of the TCE for toluene degradation than that without the TCE.

In this current study, the impact of the TCE on the toluene degradation rate constant was numerically quantified. This substance interaction feature cannot be captured by the

toluene-only kinetic model. Except for the special case of the toluene rate constant, good agreement from the different models (i.e., the single-compound models and the dual-compound models) implied that the Bayesian estimates were robust even under different modeling approaches.

The advantage of using separate simplified models for parameter estimation, as in Equations (6) and (8), is that model fitting is then easier to converge. However, the disadvantages are that (a) more experimental data need to be collected, (b) sometimes the experiments are time-consuming, and (c) in a more complicated biological system, decomposed models cannot usually capture interactions among coexisting different substrates. In such cases, using the comprehensive model is the best option for performing parameter estimation, and the Bayesian approach would provide a relatively easy way for its implementation and a reliable estimation method for high-dimensional space parameter estimation.

4.3 TCE Toxicity Effect

It was noticed that the TCE transformation capacity (T_C) that was obtained based on the data in this study applied only to the low TCE concentration range. That is, the ≥ 2 mg/L model prediction showed a relatively large deviation from the experimental data (see Figure 4 at 2 mg/L TCE and Figure 5 at 5 mg/L TCE). This may indicate that the TCE transformation capacity is a function of the TCE concentration and that the higher TCE concentration results in a more toxic effect on the biomass. This also suggests that the TCE biodegradation model may be revised to consider the impact of the TCE concentration on its own degradation, as shown in Equations (9) and (12). Li, Li, Wang, Fan, and Sun (2014) and Chen, Lin, Huang, and Lin (2008) also reported that the TCE degradation rate decreased in the presence of a high TCE initial concentration when using phenol as the growth substrate, possibly due to its self-inhibition.

5. Conclusions

A Bayesian method has been applied in this study for estimating toluene and TCE biodegradation kinetic parameters under batch experiment conditions by *Pseudomonas putida* F1. The parameters obtained from this method consistently agreed with those obtained from the traditional method where more data were needed for either toluene or TCE as a sole substrate biodegradation.

Under cometabolism (i.e., toluene degradation in the presence of TCE), the Bayesian method was able to take into account the interactions between the TCE and the toluene dioxygenase, which is responsible for the TCE and toluene degradation. In such cases, the toluene rate constant tended to be larger than that without the TCE, which numerically supported the earlier findings that TCE can induce *tod* operon for *Pseudomonas putida* F1.

Models using parameters obtained from the Bayesian method also demonstrated good prediction power on the time course of the toluene and TCE biodegradation. The model predictions had good agreement with the experimental data at various initial concentrations of interest.

Due to the TCE toxicity effect on the cells, the kinetic parameters derived from this study applied only to TCE concentrations that were 2 mg/L or lower. The TCE toxicity may have been a function of the TCE concentration, so different mathematical models may be needed when the TCE concentration is high.

This study presented a statistical approach for estimating toluene and TCE cometabolism biological parameters under local experiment conditions. The parameters were not sensitive to toluene and TCE concentrations that were below 50 mg/L and 2 mg/L, respectively. This Bayesian method with a MCMC simulation provided a robust way of parameter estimation with few data for high-dimensional parameters involved in nonlinear kinetic models. The SAS procedure PROC MCMC that was used in this study was implemented fairly easily.

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