Statistical Analysis on Trichloroethylene Degradation in a Bioreactor via a Response Surface Modeling Approach

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Abstract

Trichloroethylene (TCE), one of the most common contaminants found in groundwater, can be treated using microorganisms as the biocatalyst via cometabolism provided a primary substrate such as toluene is also present in the environment. This type of biodegradation is a relatively complex biological process, and obtaining a maximal TCE removal is a challenge due to competitive inhibition of the primary substrate and TCE's toxic effect. In this study, we evaluated the TCE treatment efficiency of a packed-bed biofilm reactor using *Pseudomonas putida* F1 as the biocatalyst and toluene as the growth substrate. TCE removal efficiency was used as the response variable, and interactions between TCE and toluene were studied in a statistical model under various operating conditions, including influent toluene and TCE concentrations and flow rates. The response surface modeling procedure was used to evaluate the performance of the biofilm reactor and to determine the optimal toluene feeding strategies under different influent TCE concentrations for achieving optimal TCE removal rates.

Key Words: Toluene and Trichloroethylene Cometabolism, Response Surface Modeling, Packed-Bed Biofilm Reactor.

1. Introduction

Trichloroethylene (TCE) is a major chemical product that has been widely used as a cleansing agent or solvent in various industries and in household products. It is a common pollutant found in U.S. contaminated groundwater (Hamid, Amin, Alazba, & Manzoor, 2014; Moran, Zogorski, & Squillance, 2007) due to leakage or improper disposal. TCE's toxicity and possible carcinogenic property (Eder, 1991; Scott & Jinot, 2011) cause great health concerns. Bioremediation is a promising biological process that uses microorganisms to break down harmful compounds to nontoxic ingredients. Treating TCE with biodegradation results in the transformation of TCE into carbon dioxide and chlorine, each of which is an ecofriendly substance (Pant & Pant, 2010).

TCE biodegradation faces a couple of challenges. First, the TCE reaction requires the presence of a primary substrate (e.g., methane for methane-oxidizing bacteria, toluene for toluene oxidizers), which induces the oxidation enzyme for both the primary substrate and the secondary substrate TCE reaction. Sharing the same enzyme used for decomposition with the primary substrate competitively inhibits TCE degradation. Second, the TCE reaction process results in a decrease in the growth rate or cell death due to its toxic effect

(Heald & Jenkins, 1994; Li & Wachett, 1992). Additionally, aerobic TCE treatment, whose degradation rate is regarded to be faster than that under an anaerobic environment in general (Mitra & Mukhopadhyay, 2016), needs sufficient dissolved oxygen in the aqueous phase so that oxygen is readily available as the final electron acceptor for the reaction to proceed. Under these restrictions, TCE biodegradation's operational conditions and bioreactor design become critical for the success of TCE treatment.

Biofilm reactors, where cells grow and attach to the surface of inertia carriers in the reactor, have been widely used in past decades for biodecontamination (Singh, Paul, & Jain, 2006, Yang et al., 2018). These types of reactors have an improved biodegradation capacity and greater tolerance to harsh pollutants because of their great abundance of biomass and longer biomass retention (Mitra & Mukhopadhyay, 2016). The biofilm reactor analyzed in this study used *Pseudomonas putida* F1 as the biocatalyst and toluene as the primary substrate for the aqueous phase TCE degradation. Besides its abundance of microorganisms in the biofilm, it features a recycled stream merged onto the influent stream, which diluted the toluene and TCE concentrations in the reactor. We expected that such reactor processing would mitigate the competitive inhibition and enhance the tolerance to TCE toxicity. Additionally, a small quantity of oxygen directly being introduced into the reactor eliminated the oxygen limitation for the TCE biodegradation.

To evaluate the performance of the biofilm reactor, we employed the response surface modeling (RSM) technique to analyze the experimental data, where RSM is a statistical method that studies not only the influences of various independent variables, but also their interactions on outcomes via graphic response surface. This approach is useful when a true function is unknown under such multivariate conditions (Kuehl, 2000). In this study, we fitted a polynomial regression equation for TCE removal efficiency as a function of input variables, such as influent toluene and TCE concentrations, to approximate the true nonlinear model. Further, we used this approach to identify an optimal reactor processing condition for TCE degradation.

2. Methods

2.1 Biofilm Reactor

The biofilm reactor used in this study was a 2.5-centimeter (cm) diameter and 60-cm long glass chromatography column packed with 4-millimeter (mm) diameter glass beads to serve as a physical support for the growth of the biofilm (Yu, 1998). The bacteria *Pseudomonas putida* F1 that formed the biofilm was used as the biocatalyst for TCE biodegradation. The growth media consisted of a minimum salt buffer (MSB) and the saturated toluene solution stream that merged with the TCE stream making the synthetic wastewater. Minimal oxygen was introduced and maintained in the column. The schematic bioreactor system is illustrated in **Figure 1**. As part of the effluent was circled back to the reactor, it flowed concurrently with the gas stream down the column. The bioreactor was operated under a trickling mode so that the biomass in the biofilm was covered by a thin layer of liquid solution that had maximum exposure to the gas phase with sufficient oxygen.

2.2 Operating Variables

The operating variables investigated include influent toluene concentration, influent TCE concentration, and total influent flow. According to the test results, a recycle rate at 27.5 milliliters per minute (ml/min) or above did not have much impact on TCE degradation. Therefore, the analysis was focused on experiments with a recycle rate fixed at around 27.5 ml/min. TCE biodegradation data used for analysis were obtained from three experiments.

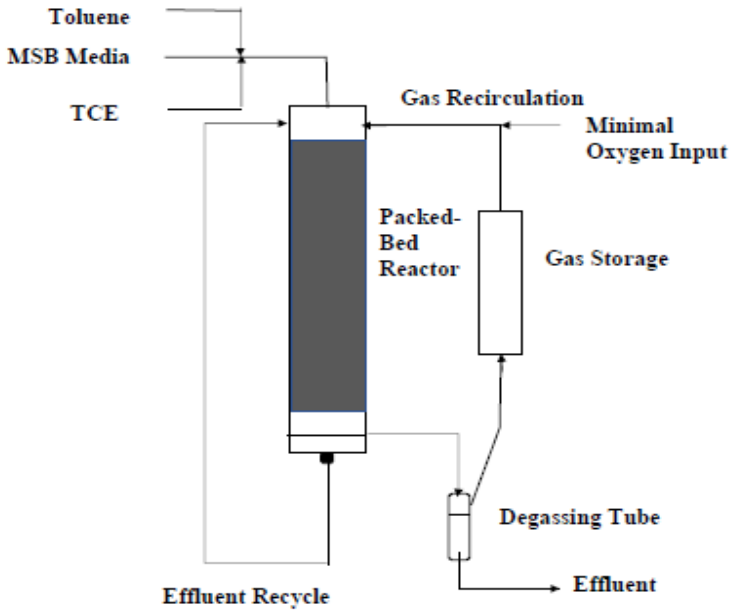

Figure 1: Biofilm reactor diagram

Experiment 1 was a test of the *influent toluene concentration* on TCE degradation for an average influent TCE concentration at (1a) 1.06 milligrams per liter (mg/L) and (1b) 1.86 mg/L, respectively. The test involved an average influent flow rate at 3.0 ml/min and a varied influent toluene concentration in the range of 9 to 69 mg/L for both (1a) and (1b) TCE concentrations.

Experiment 2 was a test of the *influent TCE concentration* on TCE degradation. The test involved an average influent flow rate at 3.1 ml/min, an average influent toluene concentration at 31 mg/L, and a varied influent TCE concentration in the range of 0.21 to 7.17 mg/L.

Experiment 3 was a test of the *flow rate* on TCE degradation. The test involved (3a) an average influent flow rate at 1.8 ml/min, an average influent toluene concentration at 53 mg/L, and varied TCE concentrations in the range of 0.14 to 2.10 mg/L; (3b) an average influent flow rate at 3.5 ml/min, an average influent toluene concentration at 28 mg/L, and varied TCE concentrations in the range of 0.36 to 2.34 mg/L; and (3c) an average influent flow rate at 4.7 ml/min, an average influent toluene concentration at 19 mg/L, and varied TCE concentrations in the range of 0.17 to 2.02 mg/L.

Besides the influent toluene and TCE concentrations, effluent TCE concertation was also measured. TCE removal efficiency, defined as % TCE biodegraded, was calculated and treated as the response variable.

2.3 Response Surface Analysis

The following second-order polynomial was used to model the outcome:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \sum \gamma_i Z_i + \varepsilon, \quad (1)$$

where Y = TCE removal efficiency (% TCE biodegraded), X_1= influent TCE concentration, X_2= influent toluene concentration, Z_1= flow rate treated as a covariate; β_0, β_i, β_{ii}, β_{ij} , γ_i = regression coefficients, and ε = random error. The data analysis was performed using SAS® 9.4.

3. Results and Discussion

3.1 Model Fitting

Experimental data were fed into SAS PROC RSREG to fit a response surface model. A fit diagnostic analysis showed that the residuals were more likely having a normal distribution (upper row of normal quantile plot and histogram of residuals in **Figure 2**), and the determination coefficient (R^2) of 0.764 indicated that 76.4% of the variation in the response could be explained by the model (lower row of predicted vs. observed plot and residual-fit spread plot indicating adequacy of fit in **Figure 2**).

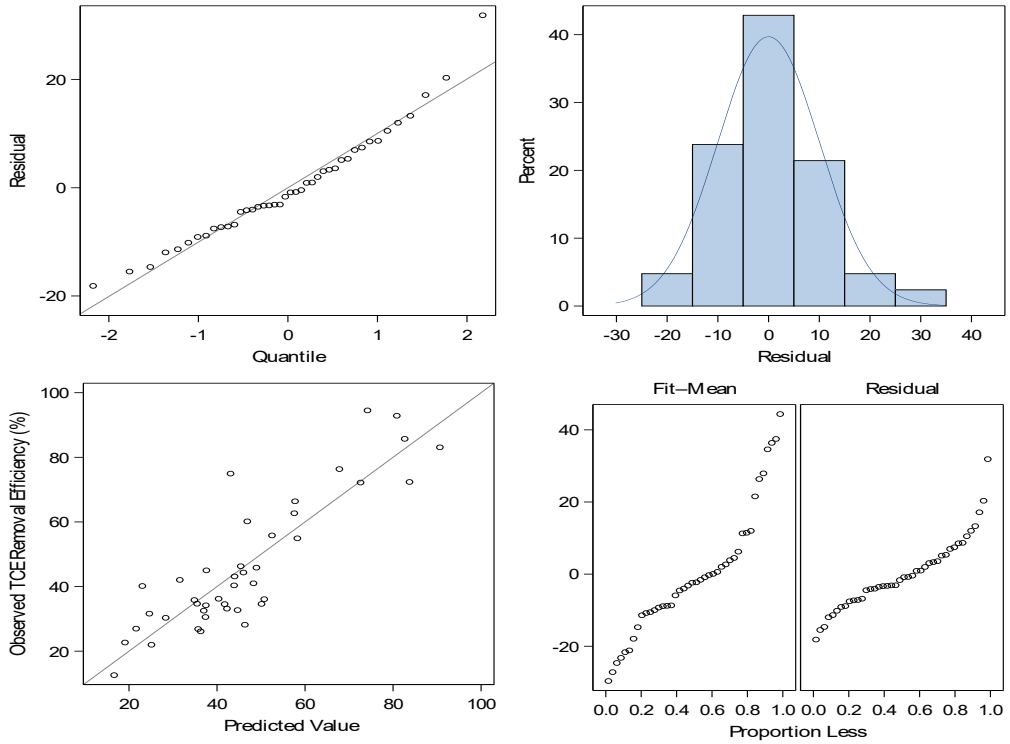

Figure 2: RSM diagnostic plots

3.2 Model Results and Validation

Analysis of variance showed that under the column operation conditions, the interaction between TCE concentration and toluene concentration (X_1X_2) was not significant with a p value at 0.7101, which means the competitive inhibition from toluene to TCE biodegradation was not significant under the column operation conditions. However, both the TCE and toluene concentrations and their quadratic terms were significant with p values of less than 0.05, which suggested that the influent toluene and TCE concentrations are the key factors for TCE biodegradation. The level of MSB flow was not significant at the 0.05 level, but marginally significant at 0.10 level with a p value at 0.0954. After eliminating the nonsignificant terms, the fitted RSM model was reduced to the following TCE degradation model:

$$Y = 73.57 - 55.62X_1 + 1.68X_2 + 12.77X_1^2 - 0.02X_2^2. \quad (2)$$

To view the impact of the factors on TCE degradation, Equation 2 was plotted in a three-dimensional scale by varying the input toluene and TCE concentrations and calculating the response variable % TCE biodegradation. **Figure 3** shows that increasing the toluene concentration enhances the TCE degradation; however, if the toluene concentration is too high, it reduces the TCE degradation. The optimal input toluene concentration is in the range of 35 to 50 mg/L. When the TCE concentration is low (e.g., below 2 mg/L), increasing the influent TCE concentration would lead to reduced TCE removal, which may be due to the toxicity effect (Wackett & Gibson, 1988) of the TCE metabolic products to the cell. A TCE removal rate over 90% can be achieved when the influent TCE concentration is 0.3 mg/L or less. These model predictions are consistent with the experimental observations.

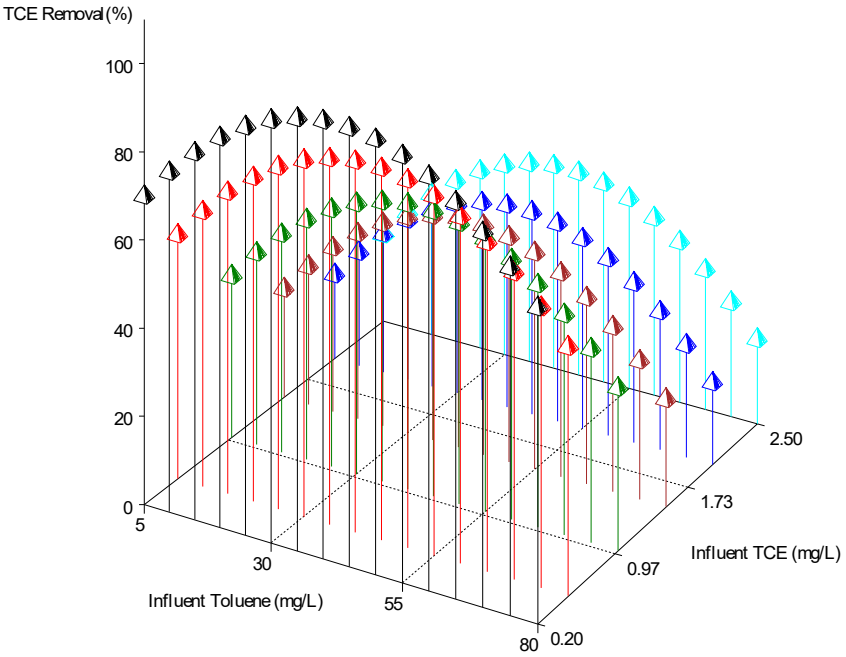

Figure 3: Correlation between % TCE removal and the influent toluene and TCE concentrations from RSM

The model was further validated by comparing the model predictions with a set of experimental data not used in the model fitting at various pairs of influent toluene and TCE concentrations. **Figure 4** shows that except for two data points—that is, (2.2, 26.9) and (1.7, 52,2)—due perhaps to uncertainties in the experiment, all other model predictions demonstrated close matches to the experimental data.

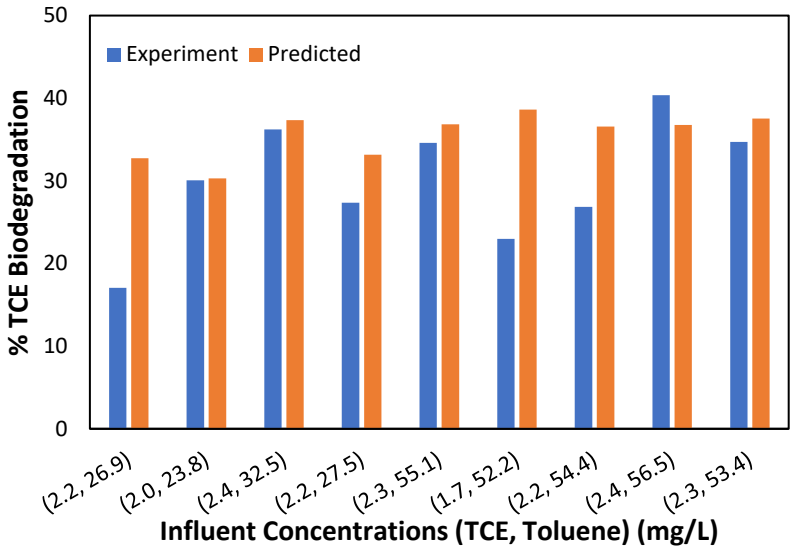

Figure 4: Comparison of experimental data and model prediction for TCE biodegradation

4. Conclusions

A response surface model was developed to evaluate the performance of a packed-bed biofilm reactor used for treating aqueous phase TCE under aerobic conditions. The model showed that the interaction between influent TCE and toluene was not significant, which numerically proved that recycling part of the effluent stream back into the react column substantially mitigated the substrate competitive inhibition to TCE degradation because of dilution. The model predicts that a maximum removal rate of greater than 90% can be achieved at low TCE concentration. The actual TCE removal efficiencies ranged from 94.5% to 60% for influent TCE concentrations from 0.31 to 1.13 mg/L, with influent toluene concentrations at 28 mg/L and 53 mg/L, respectively. The RSM model exhibited a fairly good agreement between model predictions and the experimental data that were not used in the model fitting (**Figure 3**).

The response surface analysis also showed that both inlet toluene and TCE concentrations had a significant effect on TCE removal efficiency. Considering all the effects, including competitive inhibition and TCE transformation product toxicity, the RSM predicted an optimal toluene concentration path for each of the TCE concentrations to reach a maximal TCE degradation effort. Under the experimental conditions, the model predicted that the best influent toluene concentration for TCE degradation was in the range of 35 to 50 mg/L.

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References

Eder, E. (1991). Toxicology of C_1–C_3 chlorinated hydrocarbons. *Chemosphere, 23*, 1783–1801. https://doi.org/10.1016/0045-6535(91)90026-a

Hamid, S., Amin, M. T., Alazba, A. A., & Manzoor, U. (2014). Simultaneous degradation of trichloroethylene and toluene by *Burkholderia cepacia* G4 and the effect of biotransformation on bacterial density. *CLEAN Soil Air Water, 42*, 998–1003. https://doi.org/10.1002/clen.201300201

Heald, S., & Jenkins, R. O. (1994). Trichloroethylene removal and oxidation toxicity mediated by toluene dioxygenase of *Pseudomonas putida*. *Applied and Environmental Microbiology, 60*, 4634–4637. https://www.ncbi.nlm.nih.gov/pubmed/7811103

Kuehl, R. O. (2000). *Design of experiments: Statistical principles of research design and analysis* (2nd ed.). Pacific Grove, CA: Duxbury Press.

Li, S., & Wackett, L. P. (1992). Trichloroethylene oxidation by toluene dioxygenase. *Biochemical and Biophysical Research Communications, 185*, 443–451. https://doi.org/10.1016/s0006-291x(05)81005-8

Mitra, A., & Mukhopadhyay, S. (2016). Biofilm mediated decontamination of pollutants from the environment. *AIMS Bioengineering, 3*(1); 44–59. https://doi.org/10.3934/bioeng.2016.1.44

Moran, M. J., Zogorki, J. S., & Squillance, P. J. (2007). Chlorinated solvents in groundwater of the United States. *Environmental Science & Technology, 41*, 74–81. https://doi.org/10.1021/es061553y

Pant, P., & Pant, S. (2010). A review: Advances in microbial remediation of trichloroethylene (TCE). *Journal of Environmental Sciences, 22*(1), 116–126. https://doi.org/10.1016/s1001-0742(09)60082-6

Scott, C. S., & Jinot, J. (2011). Trichloroethylene and cancer: Systematic and quantitative review of epidemiologic evidence for identifying hazards. *International Journal of Environmental Research and Public Health, 8*, 4238–4271. https://doi.org/10.3390/ijerph8114238

Singh, R., Paul, D., & Jain, R. K. (2006). Biofilms: Implications in bioremediation. *Trends in Microbiology, 14*, 389–397. https://doi.org/10.1016/j.tim.2006.07.001

Wackett, L. P., & Gibson, D. T. (1988). Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* F1. *Applied and Environmental Microbiology, 54*, 1703–1708. https://www.ncbi.nlm.nih.gov/pubmed/3415234

Yang, C., Qian, H., Li, X., Cheng, Y., He, H., Zeng, G., & Xi, J. (2018). Simultaneous removal of multicomponent VOCs in biofilters. *Trends in Biotechnology, 36*, 673-685. https://doi.org/10.1016/j.tibtech.2018.02.004

Yu, F. (1998). *Experimental design and mathematical modeling of trichloroethylene cometabolism in a packed bed biofilm reactor by* Pseudomonas putida *F1* (Unpublished doctoral dissertation). University of Tennessee, Knoxville, TN.