MODELING AND SIMULATION OF BIOREMEDIATION BY PHENOL-DEGRADING BACTERIA FOR TCE-CONTAMINATED GROUNDWATER

S. Soda [*], K. Yamaguchi [*], M. Ike [**] and M. Fujita [**]

[*] *Department of Global Architecture, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan*

[**] *Department of Environ. Eng., Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan*

ABSTRACT

A simple two-dimensional numerical model was developed to simulate the *in situ* bioremediation process against trichloroeylene (TCE) -contaminated groundwater by utilizing indigenous/exogenous phenol-degrading bacteria. The model predicts the spatial and temporal transport and fate of TCE, phenol, phenol-degrading bacteria in a confined aquifer. Convection, dispersion, and cometabolic biodegradation of TCE were considered in the model. Using the model, the cometabolic biodegradation of TCE with a variety of the injection rate of phenol and phenol-degrading bacteria into the groundwater and bacterial processes for further model development were discussed.

KEYWORDS

Modeling; simulation; bioremediation; trichloroetylene, cometabolism.

INTRODUCTION

Trichloroethylene (TCE) is one of the most hazardous soil/groundwater contaminants, which is known to persist for a long time in natural environments and suspected of being carcinogenic (Tase, 1992). TCE in soils and aquifers is not fully mobilized by flowing water, therefore, physico-chemical treatment such as pump-and -treat strategy is not always successful. Digging up of contaminated soils and their treatment or disposal is practically impossible for large area contamination. It is well known that several groups of microbes cometabolite TCE when grown upon phenol, toluene, cresol, propane, methane, and ammonia (cometabolic degradation). Biodegradation at the polluted site namely *in situ* bioremediation is an alternative for complete and cost-effective removal of TCE. *In situ* bioremediation includes two approaches, biostimulation and bioaugmentation. The former is activation of indigenous microbes, and the latter is amendment of exogenous degrading microbes capable of degradation of the target pollutants. Hopkins *et al.* (1994) reported that phenol and toluene were more effective for stimulation of indigenous bacteria for removing TCE than methane and ammonia in *in situ* field experiments. Common and abundant presence of phenol-degrading bacteria in a variety of soils and their eminent TCE-degrading capabilities in bioremdiation have been also reported (Fujita and Ike, 1997).

Numerical models for the transport of TCE within the aquifer are important in predicting its ultimate fate particularly with respect to sources of drinking water. Thus, in case of *in situ* bioremediation by the cometabolic degradation process by phenol-degrading bacteria, there is the possibility that phenol or exogenous bacteria injected into the aquifer cause the secondary pollution. Heydarpour and Slota (1989) and other researchers have developed models describing the transport of pollutants through porous media although the first-order reaction was usually assumed in the biodegradation process of pollutants in such models. On the other hand, Chang and Alvarez-Cohen (1995) and Criddle (1993) developed the

cometabolic biodegradation process. In this study, a simple two-dimensional (2-D) transport model of TCE, phenol, and phenol-degrading bacteria in groundwater is developed considering the cometabolic degradation process. Using the model, the effect of bioremediation was simulated and further model improvement was discussed.

MODEL DVELOPMENT

Transport of TCE, phenol, and microorganisms

In developing the 2-D model, following assumptions were made. The aquifer is homogenous and isotopic with respect to the transport and fate of TCE, phenol, and phenol-degrading bacteria. The aquifer thickness Δz (m) is constant and the average hydrodynamic pore velocity of groundwater U (m/s) is constant across the thickness. The y and z direction flow velocity components are zero as shown in Fig. 1. The chemicals and the bacteria are uniformly mixed through the height of the aquifer and are transported horizontally in the confined aquifer with a dispersion mechanism which occurs in the x, y direction only:

$$\frac{\partial C}{\partial t}+\frac{U}{R}\frac{\partial C}{\partial x}=Q+\frac{D_x}{R}\frac{\partial^2 C}{\partial x^2}+\frac{D_y}{R}\frac{\partial^2 C}{\partial y^2}+\left(\frac{\partial C}{\partial t}\right)^{*} \qquad \text{(Eq. 1)}$$

where C refers to the mean concentrations of TCE (C_{TCE}), phenol (C_{Phe}), or phenol-degrading bacteria (C_X) (mg/l) across the aquifer thickness. Q, R, and t are source strength (g/m^3/d), retention coefficient (-), x-direction, and time from zero point reference time (d). D_x and D_y are dispersion coefficients for x- and y-direction, respectively (m^2/d). The term $\left(\frac{\partial C}{\partial t}\right)^{*}$ refers to biochemical reaction rate (mg/l/d).

Release of TCE from polluted point

TCE is released from a polluted point with the result that its surface area S (m^2) in the aquifer varies with time according to the following equation (Muraoka and Hirata, 1990):

$$\frac{dV}{dt}=-\alpha US \qquad \text{(Eq. 2)}$$

where V and α are volume of TCE at the polluted point (m^3) and solution constant of TCE (-). The TCE source strength Q_{TCE} (g-TCE/m^3/d) can be expressed as follows:

$$Q_{TCE}=\frac{-\left(\frac{dV}{dt}\right)}{\rho_C \Delta x \Delta y \Delta z} \qquad \text{(Eq. 3)}$$

where Δx and Δy are mesh size for x and y direction for numerical calculation, respectively. ρ_c is TCE-density (g/m^3). Here, it was assumed that the mass of TCE formed a nearly globular shape i.e. V and S are expressed as functions of the radius r (m), $V = (4/3)\pi r^3$ and $S = 4\pi r^3$. Therefore, V is expressed as

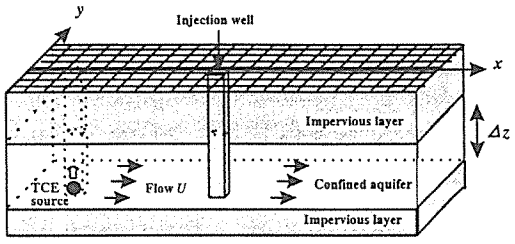

Figure 1. Leaching of TCE into groundwater.
The TCE-source and the injection well of phenol and phenol-degrading bacteria are located at (x, y) = (50, 0) and (150, 0), respectively.

follows:

$$V(t) = -\left\{\left(\frac{4}{3}\pi\right)^{\frac{1}{3}} \alpha U t - V(0)^3\right\} \qquad \text{(Eq. 4)}$$

Kinetics of cometabolism

Cometabolism results from the lack of specificity of enzymes and cofactors as shown Fig. 3. Traditional Michaelis-Menten or Monod-like expressions were applied to cometabolic biodegradation of phenol and TCE by phenol-degrading bacteria (Chang and Alvarez-Cohen, 1995).

$$\left(\frac{dC_{TCE}}{dt}\right)^{\cdot} = -r_{TCE} \frac{C_{TCE}}{C_{TCE} + K_{TCE}\left(1 + \frac{C_{phe}}{K_{phe}}\right)} C_X \qquad \text{(Eq. 5)}$$

$$\left(\frac{dC_{phe}}{dt}\right)^{\cdot} = -r_{phe} \frac{C_{phe}}{C_{phe} + K_{phe}\left(1 + \frac{C_{TCE}}{K_{TCE}}\right)} C_X \qquad \text{(Eq. 6)}$$

where r and K are maximum specific degradation rate (mg/mg-cells/d) and half saturation constant (mg/l). Subscripts *TCE* and *phe* refer to TCE and phenol, respectively. Actually, oxygen is also an important factor for the cometabolic biodgeradation but it was assumed in this study that the groundwater contains enough oxygen not to limit the biodegradation rates.

For reactions which result in product toxicity of TCE degradation, the transformation capacity can be utilized to quantify the toxic effect on the cell activity. Combining cell growth by phenol degradation with cell inactivation by product toxicity and endogenous decay, the net cell growth can be described as follows:

$$\left(\frac{dC_X}{dt}\right)^{\cdot} = -Y\left(\frac{dC_{phe}}{dt}\right)^{\cdot} + \frac{1}{T_c}\left(\frac{dC_{TCE}}{dt}\right)^{\cdot} - bC_X \qquad \text{(Eq. 7)}$$

where b and Y, and T_C are endogenous decay constant (d^{-1}), yield (mg-cells/mg-phenol), and transformation capacity (mg-TCE/mg-cells).

Figure 4 illustrates simultaneous phenol and TCE degradation at a batch condition. The TCE degradation rate is enhanced in the presence of phenol; in its absence, degradation of TCE is linked to the consumption of biomass.

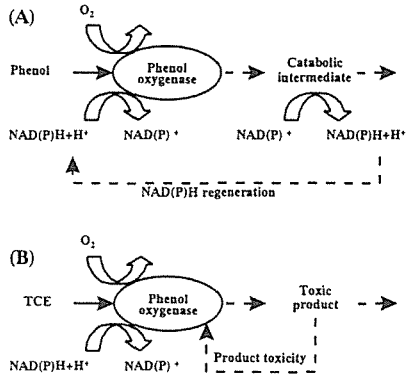

Figure 2. A typical phenol oxygenase reaction for growth substrate phenol (A) and cometabolic substrate TCE (B). The products of phenol oxidation undergo further degradations that regenerate NAD(P)H for additional substrate oxidations. The toxic products of TCE oxidations do not regenerate NAD(P)H and damage cell components.

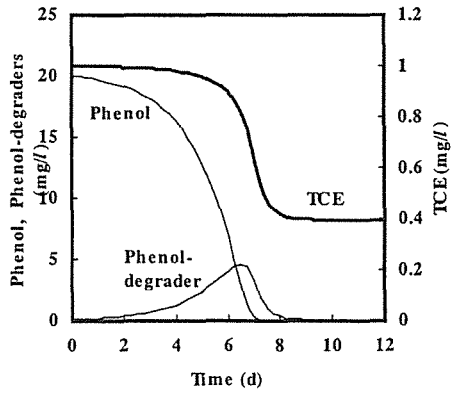

Figure 3. Cometabolic degradation of TCE in the presence of phenol in a batch culture condition. Bacterial parameters are shown in Table 1.

SIMULATION RESULTS AND DISCCUSION

Simulation scenarios

Simulation conditions and bacterial parameters used in this study are shown in Table 2. The retention coefficient R is a function of the soil characteristics (Heydarpour and Slota, 1989; Oleszkiewicz, 1993) but for simplicity it was assumed that $R_{TCE} = 2.0$ and $R_{phe} = R_X = 1.0$.

Simulation scenarios for *in situ* bioremediation, case A-C, were assumed as shown in Table 2. In all cases, the first 300 days (t = 0 - 300) were the TCE-transport period before bioremediation. TCE of 10 kg leached from a source point (x, y) = (50, 0) following with Eqs. 3 and4. The initial population density of indigenous phenol-degrading bacteria in groundwater was set at a constant value 1.0 x 10^{-4} mg/l. Spatial and temporal transport of TCE in the aquifer during the period is illustrated in Fig .4. About 1070 g of TCE leached into groundwater and about 30 g of the leached TCE was degraded. On day 300 the peak concentration of TCE showed 2.6 mg/l at the source point. The area of the TCE-polluted site over Japan groundwater quality standard (0.03 ppm) reached 3690 m^2.

Then, it was assumed that the remaining TCE at the source point (about 9 kg) was removed by some physicochemical treatments such as pumping and air stripping on day 300. From day 300 no injection of phenol nor phenol-degrading bacteria was assumed in case A (control). In cases B and C, it was supposed that an injection well of phenol and phenol-degrading bacteria was established at a point (x, y) = (150, 0). In case B, phenol was augmented at 50, 100, and 200 g/d (biostimulation). In case C, phenol-degrading bacteria were augmented at 50, 100, and 200 g/d in addition with phenol of 100 g/d (bioaugmentation).

Effect of biostimulation and bioremediation

Simulation results are summarized in Table 1. The model developed and the simulation conditions in this study were too simple for practical simulations, however, these results suggest that bioremediation is an effective purification way of TCE-polluted soil/groundwater. In practical bioremedation projects, barrier

Table 1 Simulation conditions and parameter values used in this study

b : 0.2 (d^{-1})	R_{phe}: 1.0 (-)	Y : 0.2 (mg-cells/mg-phenol)[b]
D_x : 1.0 (m^2/d)	R_X: 1.0 (-)	α : 1.47×10^{-4} (-) [b]
D_y : 0.1 (m^2/d)	r_{TCE} : 0.21 (mg-TCE/mg-cells/d) [a]	Δx: 5.0 (m)
K_{TCE} : 2.04 (mg-TCE/l) [a]	r_{phe} : 2.0 (mg-phenol/mg-cells/d)	Δy: 2.0 (m)
K_{phe} : 1.0 (mg-pehnol/l)	T_c : 0.03 (mg-TCE/mg-cells)	Δz: 1.0 (m)
R_{TCE}: 2.0 (-)	U : 1.0 (m/d)	

[a] Chang and Alvarez-Cohen (1995), [b] Muraoka and Hirata (1990), and others were assumed in this study.

Table 2 Simulation scenario for *in situ* bioremediation by using phenol for TCE-contaminated groundwater

Case	Injection rate (g/d)		TCE degraded during days 300-600 (g) [a]	Polluted area by TCE (> 0.03 ppm) on day 600 (m^2) [b]
	Phenol	Phenol-degraders		
A (Control)	0	0	14	6530
B-1	50	0	280	6220
B-2 (biostimulation)	100	0	454	6000
B-3	200	0	621	5670
C-1	100	50	558	5680
C-2 (bioaugmentation)	100	100	630	5280
C-3	100	200	697	4720

[a] There has been TCE of 1036 g in the aquifer on day 300.
[b] TCE (> 0.03 ppm) has polluted the area of 3690 m^2 on day 300.

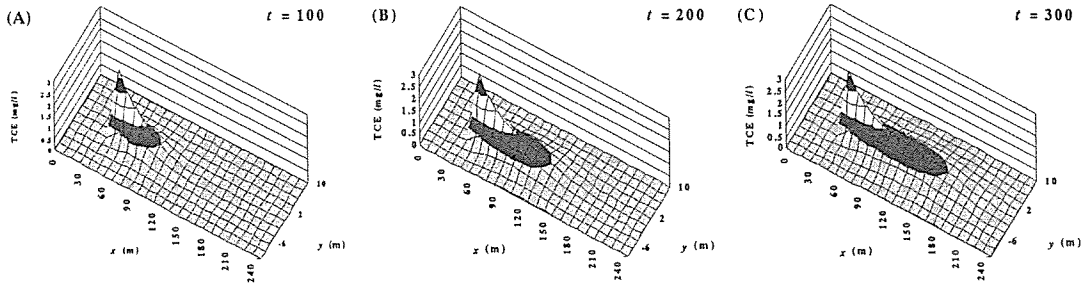

Figure 4. Spatial and temporal distribution of TCE in the aquifer on day 100 (A), day 200 (B), and day 300 (C). TCE leached continuously from the source point $(x, y) = (50, 0)$. Groundwater was polluted by 1040 g of TCE on day 300. The TCE source was removed on day 300. Effects of bioremediation after day 300 were shown in Figs 5-7. Parameters used in the simulations were shown in Table 1.

wells would be established in order to avoid the further dispersion of pollutants with injection/extraction/monitoring wells. For successful bioremediation, reasonable number and location of the wells should be determined. Injection timing and rate of substrates, nutrients, and microbes are also important for prevention of the secondary pollution.

Figure 5 shows the TCE-distribution in the aquifer in case A. The peak concentration of TCE decreased, however, the polluted area with concentration of more than 0.03 ppm spread by the diffusion mechanism. In this control case, only 14 g of TCE was degraded.

In case B, phenol-degrading bacteria stimulated by phenol-injection at the point $(x, y) = (150, 0)$. As a result, drastic TCE-degradation was shown around the injection well with increase in the phenol injection rate (Table 1). Figure 6 shows the effect of biostimulation with phenol at 100 g/d (case B-2). However, because of the small initial bacterial population and the toxic effect of TCE cometabolism, phenol over 380 mg/*l* was accumulated around the injection point on day 380 (data not shown). This result suggests that the phenol-injection rate should be controlled depending on the population and ability of the indigenous bacteria to prevent the secondary pollution by phenol. Such high concentration of phenol would inhibit the indigenous bacterial growth by its toxicity in actual conditions. Haldane-type expression describing the effect of substrate inhibition (Criddle, 1993) would be suitable for describing the growth of phenol-degrading bacteria.

In case C, further drastic TCE-degradation was computed because of wide dispersion of the phenol-degrading bacteria injected. Figure 7 shows the effect of bioaugmentation with phenol-degrading bacteria at 100 g/d and phenol at 100g/d (case C-2). Population of phenol-degrading bacteria reached 300 mg/*l* around the injection point (data not shown). Phenol concentration was almost maintained at less than 15 mg/*l*. This result suggests that bioaugmentation drastically accelerates TCE-degradation. Recently some researchers have developed desirable recombinant bacteria, which can degrade TCE without inducers such as phenol and toluene (Fujita *et al.*, 1995). There has been much concern regarding the intentional release to natural environments including soil/groundwater of such desirable bacteria for *in situ* bioremediation. However, it has been also reported that exogenous bacteria, after being injected to certain environments, drastically decreased in a variety of microcosms (Soda *et al.*, 1998). The survival of exogenous bacteria would seem to depend on their ability to tolerate a set of abiotic and biotic factors which cause lethal effects. Several abiotic factors have been suggested such as pH, temperature, and nutrients. Biotic factors are considered to include not only the growth/dacay properties of exogenous bacteria but also microbial interactions such as competition with indigenous bacteria and predation by protozoa. On the other hand, the bacterial transport process in soil/groundwater is also actually complicated. The retention coefficient R_X is usually evaluated to be 1.0 - 2.0, however, it was also reported that the presence of non-aqueous-phase

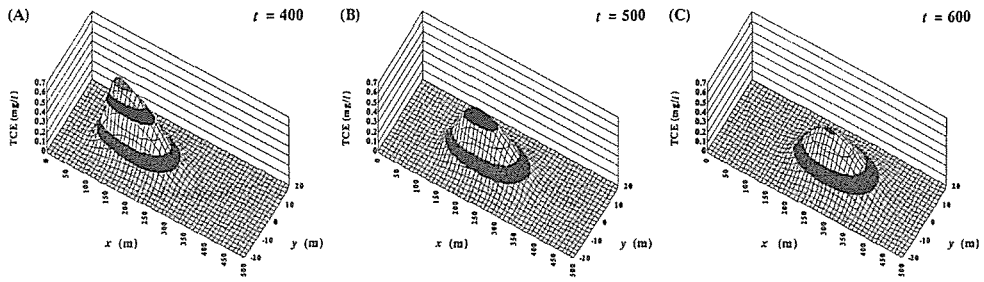

Figure 5. Spatial and temporal distribution of TCE in the aquifer without bioremediation (case A of Table 2, control) on day 400 (A), day 500 (B), and day 600 (C). During days 300-600, indigenous bacteria degraded 14 g of TCE.

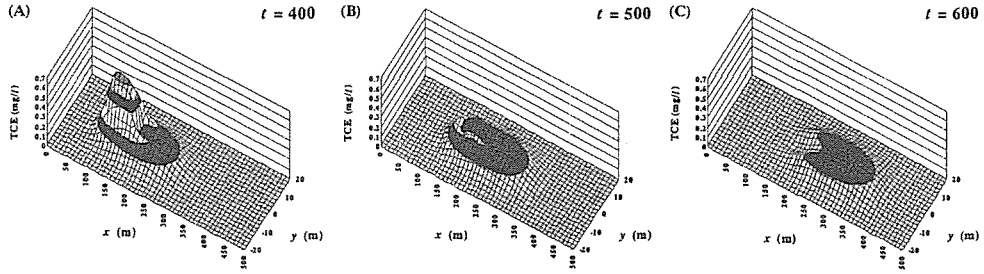

Figure 6. Spatial and temporal distribution of TCE in the auifer with biostimulation (case B-2 of Table 2) on day 400 (A), day 500 (B), and day 600 (C). Phenol was injected at 100 g/d into the point $(x, y) = (150, 0)$. Indigenous bacteria stimulated by phenol degraded 454 g of TCE during days 300-600.

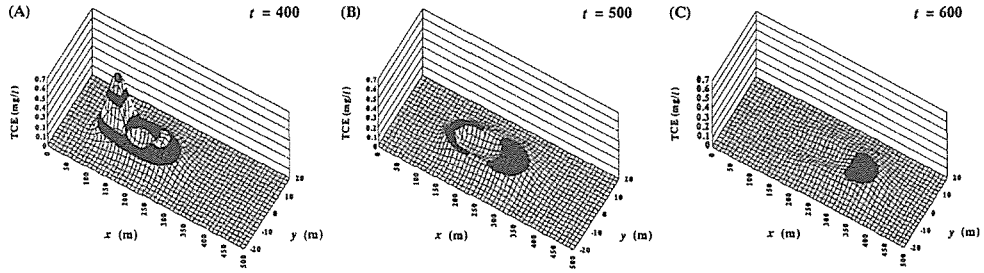

Figure 7. Spatial and temporal distribution of TCE in the aquifer with bioaugmentation (case C-2 of Table 2) on day 400 (A), day 500 (B), and day 600 (C). Phenol-degrading bacteria were injected at 100 g/d into the point (x, y) = (150, 0) with phenol of 100 g/d. Phenol-degrading bacteria mainly augmented degraded 630 g of TCE during days 300-600.

liquids (tetrachloroetylene) decreased the retention of bacteria (Rogers and Logan, 2000). Modeling such processes would be needed to improve the present model.

CONCLUSIONS

A simple 2-D model of bioremediation was developed for TCE-contaminated groundwater by utilizing indigenous/exogenous phenol-degrading bacteria. The simulation results suggested that biostimulation by phenol-injection is effective for comtabolic degradation of TCE although the initial small population of the indigenous bacteria would limitate the degradation rate. While, bioaugmentation with exogenous bacteria

was suggested to accelerate the TCE-degradation. Detailed models which consider the transport and survival proocesses of bactreia injected into soil/groundwater will be helpful for further studies.

ACKNOWLEDMENTS

This work was partly supported by a Grant-in Aid for Encouragement of Young Scientists from the Ministry of Education, Science, Sports and Culture of Japan (No. 12780423).

REFERENCES

Chang, H-L. and Alvarez-Cohen, L. (1995) Model for the cometabolic biodegradation of chlorinated organics. *Environ. Sci. Technol.,* **29,** 2357-2367.

Criddle, C. S. (1993) The kinetics of cometabolism. *Biotechnol. Bioeng.,* **41,** 1048-1056.

Fujita, M. Ike, M., Hioki, J., Kataoka, K., and Takeo, M. (1995) Tricloroetylene degradation by genetically engineered bacteria carrying cloned phenol catabolic genes. *J. Ferment. Bioeng.,* **79,** 100-106.

Fujita, M. and Ike, M. (1997) Trichloroetylene degradation by phenol degrading bacteria. *Recent. Rev. Devel. Microbiol.,* **1,** 222-343.

Heydarpour, J. and Slotta, L. S. (1989) 1-D and 2-D personal computer programs for solute transport through porous media. *Environ. Software,* **4,** 62-69.Muraoka, K. and Hirata, T. (1990) Basic study on TCEs behavior in subsurface environment. *Wat. Sci. Technol.,* **22,** 79-86.

Hopkins, G. D., Semprini, L., and McCartry, P. L. (1993) Microcosum and in situ field studies of enhanced biotransformation of TCE by phenol-utilizing microorganisms. *Appl. Environ. Microbiol.,* **59,** 2277-2285.

Muraoka, K. and Hirata, T. (1990) Basic study on TCEs behavior in subsurface environment. *Wat. Sci. Technol.,* **22,** 79-86.

Oleszkiewicz, J. A. (1993) Groundwater contamination with TCE: The problem and some solutions -A review. *J. Soil Contamination.,* **2,** 205-228.

Rogers, B. and Logan, B. E. (2000) Bacterial transport in NAPL-contaminated porous media. *J. Environ. Eng.,* **126,** 657-666.

Soda, S., Watatani, H., Ike, M., and Fujita, M. (1998) Factors affecting the survival of exogenous bacteria in microbial ecosystems: existence of indigenous bacteria with antagonistic activity. *Biocontrol Sci.,* **3,** 63-72.

Tase, N. (1992) Groundwater contamination in Japan. *Environ. Geol. Sci.,* **20,** 15-20.