Pilot studies for in-situ aerobic cometabolism of trichloroethylene using toluene-vapor as the primary substrate

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Abstract

In-situ pilot studies of aerobic cometabolism were conducted to evaluate the injection of toluene-vapor and air into TCE-contaminated aquifer. Delivery of primary substrate (toluene) in a vapor state with air enhanced the growth of indigenous toluene-utilizing bacteria that would degrade TCE by aerobic cometabolism. Meanwhile, delivering toluene in a vapor state effectively reduced potential clogging near the injection points due to excessive microbial growth, which was observed in the field when the injection of neat toluene was employed. Over 90% removal of TCE was achieved with primary substrate (toluene) degraded to a concentration below 10 μg/L.

Keywords: In-situ bioremediation; Aerobic cometabolism; TCE; Toluene; Clogging

1. Introduction

Trichloroethylene (TCE) was widely used as a solvent in the second 50 years of the 20th century. Because of uniform disposal practices for spent solvents in the past, it has become a major groundwater contaminant in Taiwan (Kuo et al., 2000). Wilson and Wilson (1985) first found that TCE might be susceptible to cometabolism by soil communities fed natural gas. Cometabolism is a process that microorganisms growing on primary substrate produce enzyme and fortuitously transform secondary substrate, from which microorganisms cannot obtain energy for growth (Little et al., 1988). Extensive research addressing aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs) by bacteria oxygenase systems has been performed. Effective cometabolic transformations of CAHs by aerobic microorganisms growing on methane (Fogel et al., 1986), phenol (Nelson et al., 1987; Nelson et al., 1988), and toluene (Wackett and Gibson, 1988) have been demonstrated. Over the past decade, both laboratory microcosm and field in-situ studies demonstrated that TCE was efficiently biodegraded by aerobic cometabolism in the presence of phenol or toluene (Hopkins and McCarty, 1995; Jenal-Wanner and McCarty, 1997).

McCarty et al. (1998) demonstrated at full scale that in-situ cometabolic degradation of TCE in groundwater through toluene injection is a viable process. However, excessive microbial growth was identified near the injection points of neat toluene. Addition of hydrogen peroxide and pulsing of toluene were employed to mitigate the clogging problems. Well redevelopment to remove bacteria clogging could be one major operational cost of the in situ bioremediation (McCarty et al., 1998). The purpose of this paper is to study the idea of injecting toluene in vapor state as a means to reduce clogging from bacteria growth around injecting points.
Delivering electron acceptors, electron donors, and nutrients in gas state to feed methane-utilizing bacteria has been reported for in situ bioremediation of TCE-contaminated sites (Brockman et al., 1995; Pfifer et al., 1997). This paper presents the results of a pilot study conducted to evaluate the feasibility of injecting toluene-vapor for: (1) enhancing the growth of indigenous toluene-utilizing bacteria that would degrade TCE by aerobic cometabolism, and (2) avoiding potential clogging near the injection points.

The majority of the research and site application of in situ bioremediation has been focused on site contaminant characterization, bacteria cultures for bioaugmentation, or the application of inorganic nutrients, electron acceptors and/or electron donors for biostimulation. Minor emphasis has been made to improve the delivery system for electron acceptors and to enhance contact and control of an electron acceptor and/or an electron donor within the contaminated subsurface soils. The field application of in situ bioremediation was relatively unsuccessful because of an ineffective distribution of electron acceptors and/or electron donors in the subsurface environment.

### 2. Materials and methods

#### 2.1. In situ pilot studies

The field experimental site is located at the Que-Jen experimental station of the National Cheng Kung University. Pilot A shown in Fig. 1 was used to: (1) determine whether toluene-utilizing bacteria could be easily biostimulated with toluene-vapor, (2) monitor whether bacteria clogging near the injection points could be prevented with the injection of toluene-vapor in the test zone, and (3) assess the kinetics and degree of TCE transformation under active biostimulation.

The pilot consisted of an injection well (A-1) and an extraction well (A-9) located 11.84 m apart, with seven monitoring wells in between (Fig. 1). Both the injection and extraction wells consisted of standard 6-in diameter polyvinyl chloride pipe with 0.5-m long screens. The monitoring wells except A-5 consisted of standard 1.25-in diameter polyvinyl chloride pipe with 0.5-m long screens. The monitoring well A-5 consisted of standard 2-in diameter polyvinyl chloride pipe with 0.5-m long screens. Seven monitoring wells...
were used to sample the ground water as it travelled through Pilot A.

The pilot was constructed with a 30 cm wide and 1 m deep trench. Impermeable liners were installed on the sidewalls and bottom of the pilot. Spargers were first installed on the bottom of the trench with a spacing of 1 m per sparger. The pilot was then filled with clean coarse sand and followed with a layer of silt about 15 cm thick and a concrete cover about 5 cm thick. Fig. 1 shows the schematic diagram and the steady-state water-table profile maintained during experiments for Pilot A. Biostimulation and biotransformation experiments were performed under induced-gradient condition by the injection and extraction of synthetic groundwater. The steady-state flow rate of synthetic groundwater in Pilot A was 170 L/day.

Residence time is defined as the saturated pore volume divided by the flow rate. The groundwater flow in Pilot A can be visualized as a plug-flow reactor. The kinetic data of Pilot A will be interpreted using residence time. Table 1 shows the location, water-table depth, saturated pore volume, and residence time for each well in Pilot A.

Toluene-vapor injection was accomplished through the use of venturi tube where the injected air vaporizes and carries toluene from a liquid-toluene reservoir located at the vena contracta of the venturi tube, as shown in Fig. 1. Toluene-vapor and air were distributed into the groundwater through spargers uniformly spaced at the bottom of Pilot A. The toluene-vapor and air were delivered through pulse injection.

The experiments were performed with the continuous injection of measured concentrations of TCE into injection well A-1 of Pilot A. The concentration history of TCE was observed using the monitoring wells in Pilot A. Table 2 presents the sequence of field experiments and processes studied. Pilot A was first biostimulated in two phases to establish a toluene-degrading consortium. Then, biotransformation experiment was performed in Pilot A.

In order to establish a toluene-degrading consortium in Pilot A, air was continuously injected for 21 days in

<table>
<thead>
<tr>
<th>Monitoring well</th>
<th>Distance from injection well (m)</th>
<th>Water table measured from wellhead (m)</th>
<th>Bottom of pilot measured from wellhead (m)</th>
<th>Saturated pore volume from injection well (liter)</th>
<th>Residence timea from injection well (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>0</td>
<td>-0.58</td>
<td>-0.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A-2</td>
<td>0.64</td>
<td>-0.62</td>
<td>1</td>
<td>24</td>
<td>0.14</td>
</tr>
<tr>
<td>A-3</td>
<td>2.45</td>
<td>-0.68</td>
<td>-1</td>
<td>97</td>
<td>0.57</td>
</tr>
<tr>
<td>A-4</td>
<td>4.75</td>
<td>-0.73</td>
<td>-1.1</td>
<td>196</td>
<td>1.16</td>
</tr>
<tr>
<td>A-5</td>
<td>5.67</td>
<td>-0.78</td>
<td>-1.19</td>
<td>231</td>
<td>1.36</td>
</tr>
<tr>
<td>A-6</td>
<td>6.67</td>
<td>-0.78</td>
<td>-1.23</td>
<td>273</td>
<td>1.61</td>
</tr>
<tr>
<td>A-7</td>
<td>8.74</td>
<td>-0.83</td>
<td>-1.25</td>
<td>344</td>
<td>2.03</td>
</tr>
<tr>
<td>A-8</td>
<td>10.91</td>
<td>-0.84</td>
<td>-1.26</td>
<td>432</td>
<td>2.54</td>
</tr>
<tr>
<td>A-9</td>
<td>11.84</td>
<td>-0.85</td>
<td>-1.27</td>
<td>466</td>
<td>2.74</td>
</tr>
</tbody>
</table>

aGroundwater flow rate: 170 L/day.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Duration</th>
<th>TCE steady-state influent concentration (µg/L)</th>
<th>Pulse of toluene-vapor injection (g)</th>
<th>Pulse of air injection# (SCF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biostimulation</td>
<td>1-23-2003<del>2-17-2003 (Day 1</del>Day 26)</td>
<td>430±119</td>
<td>0~50</td>
<td>90~330*</td>
</tr>
<tr>
<td></td>
<td>2-18-2003<del>3-17-2003 (Day 27</del>Day 54)</td>
<td>430±119</td>
<td>60</td>
<td>270*</td>
</tr>
<tr>
<td>Biotransformation</td>
<td>3-18-2003<del>4-24-2003 (Day 55</del>Day 92)</td>
<td>430±119</td>
<td>60</td>
<td>270*</td>
</tr>
</tbody>
</table>

*Average rate of air injection: 9 SCF/min.
**One pulse of injection per day.
#One pulse of injection every 3 days.

Table 1
Physical data of Pilot A

Table 2
Experiments and operating conditions of Pilot A
order to provide an aerobic environment for the toluene injection. Procedures to stimulate toluene-oxidizing bacteria were similar to those presented by McCarthy et al. (1988) except toluene-vapor instead of neat toluene was injected. Toluene-vapor was injected with air through venturi tube as shown in Fig. 1. The toluene-vapor injection schedule during the first phase of biostimulation period from 1-23-2003 to 2-17-2003 in Pilot A was as follows: 8.7 g/day from 1-23-2003 to 1-26-2003; 34.8 g/day on 1-27-2003; 17.4 g/day on 1-28-2003; no toluene-vapor injection from 1-29-2003 to 2-09-2003; 30.5 g/day on 2-10-2003; 52.2 g/day on 2-11-2003; no toluene-vapor injection from 2-12-2003 to 2-16-2003; 52.2 g/day on 2-17-2003. The bacteria counts of groundwater samples from Pilot A increased up to $10^6$ CFU/mL on 2-17-2003. During the biostimulation experiment of Pilot A, influent flow rate and TCE concentration of synthetic groundwater were kept constant at 170 L/day and 430 ± 119 mg/L, respectively. The purpose was to develop a toluene-degrading population in the presence of TCE. During the second phase of biostimulation from 2-18-2003 to 3-17-2003, toluene-vapor and air for each pulse of injection were kept constant at 60 g and 270 SCF, respectively.

After establishing a toluene-degrading consortium, biotransformation experiment was performed in Pilot A to reach steady-state operation and to assess the kinetics and degree of TCE transformation under active biostimulation. During the experiment of biotransformation at Pilot A, toluene-vapor and air for each pulse of injection were kept constant at 60 g and 270 standard cubic feet (SCF), respectively. One pulse was injected in every 3 days and the duration of each pulse averaged 30 min. The very short duration of air injection compared to the long duration of biotransformation was to minimize TCE removal by air stripping and to maximize TCE removal by biotransformation.

The experimental methodology requires controlled chemical addition to the test zone over extended periods and frequent monitoring of chemical concentrations, dissolved oxygen, and active microbial population. The performance of the injection and the analytical systems is indicated in Table 2, where the influent concentrations of TCE and their standard deviations are presented. The coefficients of variation of the injection concentrations of TCE for Pilot A was 27%. This precision permits accurate estimates of the degree of transformation.

2.2. Analytical methods

Dissolved oxygen (DO) was measured by a DO probe (YSI 5010). The pour-plate method was used for the enumeration of the active microbial population of groundwater samples. TCE and toluene were measured using an hp 6890 gas chromatograph with a J&W Scientific DB 1, 60 m, 0.32 mm i.d. capillary column, and hp 5973 MSD. Zero-grade helium carrier gas flow rate was 25 mL/min at a head pressure of 4.24 psig. The temperatures of injector and detector were 200 °C and 265 °C, respectively.

A purge and trap injection system (Tekmar Model ALS 2016 sampler connected to a Tekmar Model LSC 2000 concentrator fitted with a Tekmar Purge Trap K) was used. Samples of 5 mL were added to sampling tubes and purged for 11 min at 35 °C. Desorption preheat was at 265 °C and desorption time was 4 min at 270 °C. The trap lines and valves were kept constant at 150 °C and the injection port was 265 °C. The detection limits for TCE and toluene were 2.6 and 1.1 μg/L, respectively.

3. Results of the field experiments

3.1. Biostimulation experiment of Pilot A

Biomass distribution throughout Pilot A was evaluated by bacteria enumeration of groundwater samples during the second phase of biostimulation. Fig. 2 shows that the bacteria counts of groundwater samples in Pilot A increased from $10^4$ CFU/mL on 2-17-2003 to $10^6$ CFU/mL on 2-25-2003 and 3-10-2003. At the end of the biostimulation experiment, the bacteria counts of groundwater samples from Pilot A increased to a level above $10^6$ CFU/mL on 3-17-2003. An adequate population had been established in Pilot A to proceed to the next stage, biotransformation experiment. During the biotransformation experiment, the bacteria counts of groundwater samples from Pilot A ranged from $10^6$ to $10^8$ CFU/mL.

Samples from various monitoring wells were also analyzed for toluene and oxygen decrease. Fig. 2 also shows the dissolved-oxygen (DO) concentration monitored several days after air and toluene injection and just before the next injection of air and toluene. Fig. 2 also compares the DO concentration at various stages of biostimulation. Notice that the DO concentration was the highest on 1-26-2003 at the beginning stage; the DO data on 2-19-2003 represented the intermediate stage; the DO concentration was the lowest on 3-19-2003 at the end of biostimulation experiment. DO decrease at various stages of biostimulation provided evidence for the growth of an indigenous toluene-oxidizing population. The influent DO concentration was 9.35 ± 0.99 mg/L. The concentration of DO observed in Pilot A at the end of biostimulation experiment ranged from 0.61 to 2.52 mg/L. DO supplied from the influent groundwater and injected air were consumed by an indigenous toluene-oxidizing population established in Pilot A.

Fig. 3 shows the toluene concentration monitored several days after toluene injection and just before the next injection of toluene. Because of the pulsing
Fig. 2. Biomass and dissolved oxygen in monitoring wells of Pilot A.

Fig. 3. Toluene concentration monitored several days after toluene injection and just before the next injection of toluene in Pilot A.
injection used, toluene concentrations at the monitoring wells varied considerably with time. Toluene concentrations higher than 100 µg/L were measured for groundwater samples taken right after pulse injection of toluene. A significant decrease in toluene concentration was observed at every monitoring well several days after toluene injection and just before next pulse injection of toluene. Fig. 3 shows that toluene of each pulse injection was practically removed to a concentration below 10 µg/L. Toluene decrease everywhere in Pilot A also provided evidence for the establishment of an indigenous toluene-oxidizing population.

3.2. Biotransformation experiment of Pilot A

In this phase from 3-18-2003 to 4-24-2003 (day 55 to day 92), effort was made to achieve steady-state operation of Pilot A. During this period, toluene-vapor and air for each pulse of injection were kept constant at 60 g and 270 SCF, respectively. One pulse was injected in every 3 days and the duration of each pulse averaged 30 min. Influent flow rate and TCE concentration of synthetic groundwater were kept constant at 170 L/day and 430 ± 119 µg/L, respectively.

TCE removal: Fig. 4 presents TCE response at monitoring wells of Pilot A from 2-18-2003 to 4-24-2003. TCE concentrations at monitoring wells A-1 and A-2 were fairly steady at 430 ± 119 and 329 ± 56 µg/L, respectively from 2-18-2003 to 4-24-2003. TCE concentration at monitoring well A-3 from 3-17-2003 to 4-24-2003 was also fairly steady at 253 ± 25 µg/L. Toward the end of biotransformation experiment, TCE concentrations at monitoring wells A-4, A-5, A-6, A-7, A-8, and A-9 were at 140 ± 19, 39 ± 7, 18 ± 11, 5 ± 2, 3 ± 1, and 4 ± 3 µg/L, respectively.

Biomass distribution: Biomass distribution throughout Pilot A was evaluated by bacteria enumeration of groundwater samples at various stages during the experiment of biotransformation. Fig. 2 showed that the bacteria counts of groundwater samples in Pilot A were maintained relatively steady ranging from 10^6 to 10^8 CFU/mL with a median of 10^7 CFU/mL during the period of biotransformation experiment from 3-17-2003 to 4-21-2003. Fig. 2 also indicated that the biomass distribution of groundwater samples was fairly uniform along the plug flow reactor in Pilot A at any time during the period from 3-17-2003 to 4-21-2003. An improved distribution of air and toluene in vapor state through spargers uniformly spaced at the bottom of Pilot A was instrumental for a fairly even distribution of biomass in groundwater phase.

Toluene-vapor injection head: Toluene-vapor injection head was monitored throughout the biotransformation experiment in order to detect the problem of bacteria clogging near the injection points. Fig. 5 presents air injection pressure and flow rate at toluene-vapor delivery system in Pilot A throughout the study period. Notice that while the injection pressure was maintained constant, the flow rate of air remained fairly constant. This indicated that the problem of bacteria clogging near the injection points did not occur. Delivering toluene in vapor state was effective to prevent the injection points from bacteria clogging. The increase in pumping heads as a result of biomass buildup near the injection well of neat toluene was reported by McCarty et al. (1998). Fig. 2 shows that the progress of establishing toluene-utilizing bacteria in Pilot A. At any time during the experiments, the bacteria counts in groundwater phase were fairly uniform along the plug flow reactor of Pilot A. Instead of clogging the injection

![Fig. 4. TCE concentration monitored in Pilot A.](image-url)
points, the biomass was fairly even distributed along the plug flow reactor in Pilot A.

4. Discussion

The performance of TCE concentration of Pilot A in Fig. 4 can be used to evaluate the kinetics of TCE removal for cometabolic biotransformation. In Fig. 4, TCE removal decreased with time over several weeks of steady-state feed and aeration. This was because of sorption of TCE to the soil. The TCE concentration profile along the plug flow direction observed in Pilot A for a given day was plotted versus residence time on semi-log paper and was least-squares fitted with a first-order kinetic model. Table 3 shows the regressed rate constants of TCE removal for Pilot A. Values of sample correlation coefficients shown in Table 3 also suggest that the assumptions of first-order reaction model and the plug-flow model are appropriate.

Fig. 6 shows the variation of the first-order rate constant with the pore-volume of injection for Pilot A. Notice that the first-order rate constant decreased with an increase in the pore-volume injection for Pilot A until the sorption was completed. The sorption was completed at 26 pore-volumes of injection. After the sorption was completed, the first-order rate constant of TCE removal stabilized at 2.11 L/day.

Monod model (1949) was used to express the rate of cometabolism as follows (Jenal-Wanner and McCarty, 1997):

\[
- \frac{dS}{dt} = \frac{k_s S}{K_s + S} X_a, \tag{1}
\]

\[
- \frac{dC}{dt} = \frac{k_c C}{K_{sc} + C} X_a, \tag{2}
\]

where \( S \) (mg/L) and \( C \) (µg/L) are the primary substrate and contaminant (TCE) concentrations; \( X_a \) (mg/L) is the active biomass concentration; \( k_s \) (day\(^{-1}\)) and \( k_c \) (day\(^{-1}\)) represent the maximum rate of primary substrate and contaminant consumption per unit of active biomass per day; and \( K_s \) (mg/L) and \( K_{sc} \) (µg/L) are the affinity constants for primary substrate and contaminant, respectively. When the contaminant (TCE) concentration is low, Eq. (2) can be approximated as follows:

\[
- \frac{dC}{dt} \approx \frac{k_c}{K_{sc}} X_a C = k' X_a C \quad \text{when} \ C \ll K_{sc}. \tag{3}
\]

If \( X_a \) is uniformly distributed in the reactor, Eq. (3) can be integrated for TCE removal over a plug flow reactor as follows:

\[
C = C_i e^{-k' X_a t}, \tag{4}
\]

where \( C_i \) is the influent concentration of TCE (µg/L) and \( C \) is the concentration of TCE (µg/L) at any location with a residence time \( t \) (day) in a plug flow reactor. Eq. (4) implies that when TCE concentration is plotted versus residence time on semi-log paper, one could get a straight line with an intercept of \( C_i \) and a slope of \(-k' X_a\).

Toward the end of the biotransformation experiment, Pilot A was approaching a steady-state from April 14 to April 24, 2003. The near steady-state TCE concentration profile obtained in Pilot A was plotted versus residence time on semi-log paper and was least-squares fitted with a straight line as shown in Fig. 7. The regressed equation for the biotransformation experiment in Pilot A was as follows:

\[
\ln C = -2.039t + 6.295 \quad \text{(5)}
\]
A good value of sample correlation coefficient, $R^2=0.905$, suggests that the first-order kinetic model is appropriate for the rate equation of cometabolic degradation and that the plug-flow model is valid for Pilot A. The first-order rate constant of cometabolic degradation, $kX_a = 2.039$ day$^{-1}$ for the operating conditions in Pilot A with an active bacteria count of groundwater samples at about $10^7$ CFU/mL.

The performance of TCE concentration in Fig. 4 can be used to evaluate the efficiency of TCE removal. A percentage of TCE removal of 90.9% was observed at Well A-5 toward the end of biotransformation experiment, which is in an excellent agreement with 93.8% removal efficiency calculated by Eq. (6) using a residence time of 1.36 days for Well A-5. During the biotransformation experiment, only one pulse of toluene-vapor and air was injected every 3 days. In addition, the duration of each pulse was short and averaged 30 min. The duration of air injection was only 0.7% of that of biodegradation. The observed TCE removal in Pilot A was mainly due to biotransformation.

### 5. Conclusions

This paper investigated and proved the idea of introducing toluene vapor into groundwater as a means to reduce clogging from bacteria growth during

$$ C = 542e^{-2.039t}. \quad (6) $$
aerobic cometabolic degradation of TCE. Clogging was a problem encountered near well screens when neat-toluene was applied. The following conclusions are based on the results of the data from a 92-day pilot study consisting of biostimulation and biotransformation experiments.

1. An indigenous toluene-utilizing biological population was developed in the presence of TCE via pulse-injection for toluene-vapor and air.

2. Bacteria clogging near the injection points could be prevented using pulse-injection for toluene-vapor and air. The method of delivering primary substrate (toluene) and electron acceptors (air) in vapor or gas state appeared to improve the distribution of toluene-vapor and air in the contaminated aquifer, which was instrumental to the improvement of the distribution of biomass in the contaminated aquifer and reduction of bacteria clogging near the injection points of neat toluene.

3. Over 90% removal of TCE was observed within a 6-m bioactive zone with a hydraulic residence-time of 1.36 days. Toluene (primary substrate) was degraded to a concentration below 10μg/L.

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References


