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Long-term monitoring and evaluating biological activity of in situ anaerobic reductive dechlorination at a highly recharged and TCE-contaminated aquifer

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ABSTRACT

Few studies have evaluated how rainfall recharges affect biological dechlorination of trichloroethene (TCE). In this study, a sequence of three well-to-well tests (Phases I, II, and III) were performed for 100 d to investigate the variation of biological dechlorination of TCE at a highly recharged and TCE-contaminated aquifer. Test solutions containing different nutrients were delivered into the aquifer through a nitrogen gas spray. In Phase I, where only formate was supplied, the degradation of TCE to cis-dichloroethene (cis-DCE) was negligible. However, when sulfate as well as formate was introduced during Phase II, the reductive dechlorination of TCE occurred. During Phase III, fumarate was also provided as an alternative electron donor. High cis-DCE production rates were observed with complete degradation of formate and sulfate at 65th day monitoring, the end of dry period in Phase III. Since then, there were heavy rainfalls that accounted for approximately 36% of total annual precipitation in this area. Although oxygenated rain was rapidly infiltrated into the bioactive zone, the consumption of formate and sulfate were not significantly affected, with the DO as low as <0.2 mg/L. Nonetheless, the dechlorination activity of TCE was decreased as evidenced by 70% reduction in the cis-DCE production rate compared to that before the heavy rainfall. In 10 d after heavy rainfall ceased, the dechlorination activity was partially recovered (approximately 60%), while sulfate and formate consumptions were fully recovered. These results suggest that the dechlorination activity of TCE at the tested aquifer was sustained even in the presence of heavy rainfall recharges. This was probably due to the growth of the dechlorinating microbes on solid surfaces by forming biofilms.

Keywords: Anaerobic reductive dechlorination; TCE; Rainfall Intensity; Liquid–gas mixture spray; Well-to-well test

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1. Introduction

Biological anaerobic reductive dechlorination (ARD) of trichloroethene (TCE) and perchloroethylene (PCE) has been widely studied by many researchers. Bouwer and McCarty [1] discovered the ability of anaerobic microbes in degrading TCE and PCE. Several researchers observed transformation byproducts of PCE and TCE in the anoxic condition aquifer in the 1980s [2,3].

In the early 1990s to mid-1990s, researchers reported that dehalorespiring bacteria were able to grow on chlorinated aliphatic hydrocarbons (CAHs) for a direct energy source [4] and the rate of the metabolic ARD process for CAHs was much higher than that of the co-metabolic process [5–8]. In the early 1990s to late 1990s, a lot of research was conducted in order to recognize the effects of the reductive dechlorination activity of CAHs under a variety of different oxidation–reduction conditions [9–15].

At the beginning of the twentieth century, scientists found that hydrogen was the ultimate electron donor (ED) and that dechlorinating micro-organisms could utilize lower concentrations of hydrogen than anaerobic organisms such as sulfate-reducing bacteria and methanogens [7,16,17]. Also, they discovered that *Dehalococcoides* strains could transform TCE and PCE to innocuous products and complete biological reductive dechlorination of TCE and PCE had not occurred at CAHs-contaminated sites where *Dehalococcoides* strains did not reside [18].

In general, strictly anaerobic conditions are required for the growth and control of *Dehalococcoides* strains because they are highly sensitive to oxygen [7,16,19]. Several laboratory studies reported that a short period of air and/or oxygen exposure was extremely harmful to *Dehalococcoides* activity. That is, a short period of the exposure would inactivate the *Dehalococcoides* [20–22]. In the laboratory studies, Amos et al. [23] explored the effects of oxygen on *Dehalococcoides'* viability and its TCE dechloriation activity when oxygenated surface water infiltrates the active TCE dechlorination zone. Reductive dechlorination activity of PCE and TCE to cis-1,2-dichloroethylene (cis-DCE) appeared to recover when oxygen was removed; however, the reductive dechlorination activity of vinyl chloride (VC) to ethene (ETH) did not appear at all or took a longer period of time to recover [23].

The effects of oxygen on dechlorinating activity have not been thoroughly studied in the field. Many studies on TCE dechlorination advised against the exposure of *Dehalococcoides*-containing consortia or ARD indigenous micro-organisms to oxygen during the bioremediation of a TCE-contaminated aquifer. In the in situ enhanced bioremediation of a TCE-contaminated aquifer, preventing the oxygen exposure to ARD indigenous micro-organisms is not possible and its effects on TCE ARD is unknown. For example, infiltration of rainfall and migration of oxygenated groundwater to an ARD active zone cannot be controlled during in situ enhanced bioremediation. Recharged groundwater causes increased levels of oxygen and redox potentials in an aquifer. Few field studies have demonstrated the effectiveness of TCE reductive dechlorination at highly recharged aquifer by rainfall infiltration.

Therefore, for successful bioremediation of a highly recharged and TCE-contaminated aquifer, an evaluation of the change in dechlorination activity over a long period of time is necessary. Thus, the objective of this study is to investigate the variation in TCE dechloriation activity under the condition of continuous oxygenated groundwater recharge into a dechlorination active zone at a variety of rainfall intensities. In this study, three sequential phases of well-to-well field tests were performed during a 100 d period to investigate the objectives described above.

2. Materials and methods

2.1 Geological and hydrogeological site characterization

The well-to-well test was performed in two monitoring wells (SKW-5 and SKW-4) at the Road Administrative Office (RAO) of Gangwon Province in the Woosan Industrial Complex (WIC), Wonjoo, South Korea. The ROA aquifer has been known as a TCE-DNAPL contaminated aquifer. Also, extensive analysis of sampled soils and groundwater showed that the hot source of TCE existed upgradient of monitoring wells near the mountain [24–27]. RAO is located upward from the WIC and is surrounded by a forest hill which is known to be the main source of TCE. Therefore, for the field tests, installation of groundwater wells, and groundwater sample analyses were conducted at the test sites. Downgradient SKW-4 and upgradient SKW-5 wells were drilled to a maximum depth of 30 m. Groundwater levels of two monitoring wells were approximately 12 m below ground surface; the seasonal average hydraulic gradient and hydraulic conductivity of SKW-5 to SKW-4 were 0.007 m/m and 4.17×10^{-4} cm/s, respectively. Geological data of SKW-4 were clayey sand (below surface, 0–4 m), silty sand (4–18 m), gravelly sand (18–25 m), weathered rock of granite (25–27 m), and soft bedrock of granite (27–30 m). The SKW-5 well contained clayey sand (below surface, 0–4 m), silty sand (4–12 m), gravelly sand

Table 1
Background and injected solute composition and concentration (average concentration ± 95% confidence interval)

Sample		Elapsed time (d)	Average TCE conc. (μmol/L)	Average cis-DCE conc. (μmol/L)	Average bromide conc. (mmol/L)	Average formate conc. (mmol/L)	Average sulfate conc. (mmol/L)	Average fumarate conc. (mmol/L)
Test solution injected	Phase I	0–15	[a]N.D.	N.D.	2.87 ± 0.14	2.20 ± 0.28	[b]N.I.	N.I.
	Phase II	25–50	N.D.	N.D.	2.60 ± 0.16	2.07 ± 0.21	0.68 ± 0.07	N.I.
	Phase III	>50	N.D.	N.D.	2.60 ± 0.18	1.90 ± 0.14	0.49 ± 0.18	1.72 ± 0.08
[c]Background groundwater		0	53.4 ± 21.8	N.D.	N.D.	N.D.	N.D.	N.D.

[a]Not detected.
[b]Not injected.
[c]Average concentration for 3 years (2010–2012).

(12–25 m), weathered rock of granite (25–27 m), and soft bedrock of granite (27–30 m).

Precipitation data of Wonju meteorological station located 4 km from the RAO were used to interpret rainfall intensity at the field test site. In the last four years (2010–2013), the average annual rainfall at the research area was observed to be 1,632 mm, and approximately 75% of the annual precipitation occurred during the rainy season from June to September. During the rainy season, the study area's groundwater level fluctuated from 2 to 4 m due to rainfall recharge. The reason for the fluctuating groundwater levels in the study area is that it is surrounded by sloped forests that have high groundwater recharge potentials [26,28]. For three years (2010–2012), the average concentration of TEC, dissolved oxygen (DO), and oxidation–reduction potential (ORP) were 53.4 ± 21.8 μmol/L, 5.6 mg/L, and + 208 mV, respectively. Table 1 shows the background concentrations of solutes, solvents, and injected solution composition.

2.2. Well-to-well test

Three sequential phases of well-to-well tests were carried out to evaluate TCE dechloriation activity under the conditions of continuous oxygenated groundwater introduction into a dechlorination active zone. The three sequential phases of well-to-well tests were as follows: (1) Phase I test (Biostimulation test) was performed to stimulate indigenous TCE dechlorinating microbes by injecting formate as an ED and bromide as a tracer into a well of SKW-5; (2) Phase II test (Sulfate injection test) was carried out to assess the effects of sulfate injection on the TCE dechlorinating activity by injecting formate, bromide, and sulfate as an alternative EA; and (3) Phase III test (Activity test under high recharge condition) was conducted to evaluate the effects of rainfall recharge on TCE dechlorinating activity, and to assure the biological TCE degradation to cis-DCE by adapting fumarate as an indicator of TCE dechlorination activity.

The tests were performed through recirculation systems at two monitoring wells (Fig. 1). The distance between SKW-5 upgradient well and the SKW-4 downgradient well was 3 m and groundwater was extracted from the SKW-4 well and reinjected into the SKW-5 well. At the SKW-5 well, upper and lower inflatable packers were installed to isolate the depth interval of 20–22 m for injection. At the SKW-6 well, an upper inflatable packer was installed at 18 m for extraction.

Field tests applied a new injection method of a liquid/gas mixture spray. The test solution and nitrogen

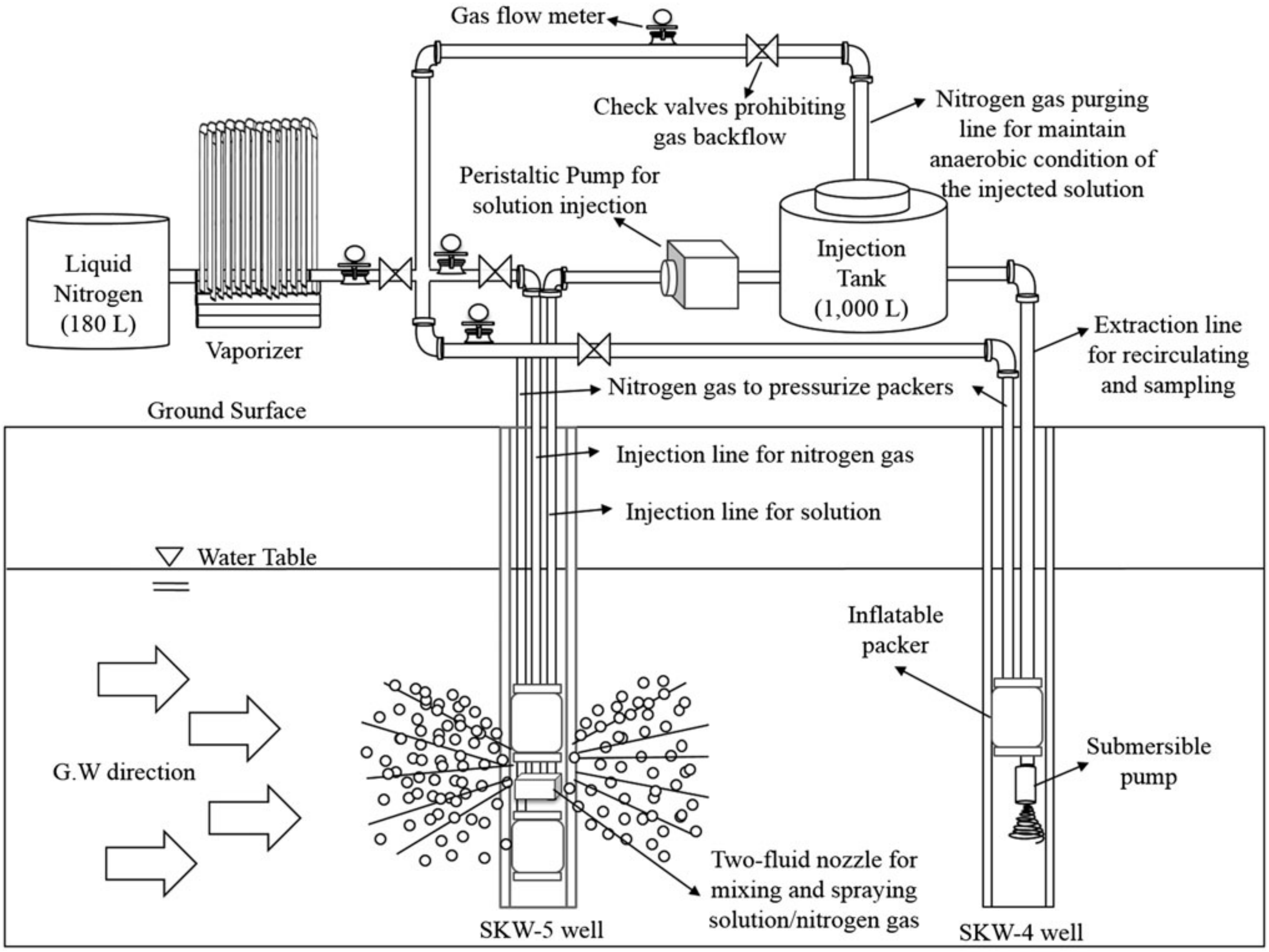

Fig. 1. Schematic of field well-to-well test setup.

gas were simultaneously and separately injected into the aquifer in the form of a spray using a two-fluid nozzle (Fig. 1). In the injection method, liquid nitrogen was injected as a gaseous form through a vaporizer. To maintain the anaerobic condition of the test solution, nitrogen gas was purged consistently during the injection period.

The test solution was injected at a rate of 1.0 L/min and nitrogen gas was simultaneously injected at a rate of 5 L/min. Groundwater was extracted at a rate of 0.5 L/min. The injected solution contained known concentrations of Br^- using potassium bromide (KBr; Spectrum Chemical Mfg. Corp. Gardena, CA) as a nonreactive tracer, and formate using sodium formate (HCOONa; Spectrum Chemical Mfg. Corp. Gardena, CA) as an ED and/or carbon source (CS). And sulfate was injected to reduce the redox potential as an alternative EA after 20 d of injection. In each phase, final concentrations of bromide, formate, fumarate, and sulfate in the test solution are presented in Table 1.

To determine the concentrations of the injected solutes, one sample (triplicate) per 1,000 L injected test solution was collected using a 40 mL VOA vials for volatile organic analysis fitted with a Teflon-lined cap and 50 mL conical tube for ion and organic acid analysis. At the same time, geochemical data of DO, ORP, EC, pH, and temperature were determined at the field. During the tests of Phase I and II, one sample was collected in a 40 mL VOA vial and a 50 mL conical tube every 12 h. Geochemical data were determined from an extracted mixture of test solution and native groundwater. During the test of Phase III, one sample was collected every day. The data of the injected and extracted sample analysis showed breakthrough curves for each injected solute.

2.3. Solute analysis

Parameters (DO, ORP, EC, pH, and temp) were measured by an YSI (OH, USA) ProPlus multiparameter equipped with a quarto-cable and flow-cell. While measuring water parameters, groundwater was continuously extracted and transported into the flow-cell. The instrument was calibrated every week.

Concentrations of TCE, and its byproducts in groundwater samples were measured by headspace gas analysis using Shimazu GC-17A (Kotyo, Japan) equipped with a flame ionization detector. The GC system was operated at 129 kPa system pressure, an 250℃ injection port temperature, a 250℃ detector temperature, and a 40–220℃ oven temperature. Chromatographic separations were performed by a GS-Gaspro 60 m × 0.32 mm column (Agilent Technologies, CA, USA). TCE (99.5%), cis-DCE (97%), trans-DCE (98%), 1,1-DCE (99.9%), VC (99.5%) and ETH (99.99%) were obtained from Sigma–Aldrich (MO, USA). A 10 mL-sample was removed from a 40 mL-VOA vial and the vial was equilibrated by a shaking incubator at 20℃. After equilibrating the sample, 200 μL of gas sample was taken using a gastight syringe and was injected into the GC.

The concentrations of anions (Br^- and SO_4^{2-},) were measured by IC equipped with a conductivity detector. Samples were pre-filtered using a 0.35 μm syringe filter and added into a Dionex AS-DV autosampler (Dionex Co., CA, USA). The IC system was operated with a flowrate of 1.2 mL/min, a mixture of 0.371 g/L sodium carbonate, 0.084 g/L for mobile phase, and a flowrate of 1.2 mL/min for mobile phase. Chromatographic separations were performed by a Dionex IonPac AS14 column (Dionex Co., CA, USA). Potassium bromide (99%) and sodium sulfate (98%) were obtained through Samchun Chemical (Gyeonggi-do, Korea). Concentrations of formate, fumarate, succinate, and acetate were determined by high-performance liquid chromatography (HPLC). Samples were pre-filtered using a 0.35 μm syringe filter and injected into a Shiseido SI-2 HPLC. The HPLC was operated with a 210 nm detector wavelength, a 37℃ oven temperature, 20 mM phosphoric acid for mobile phase, and a 600 μL/min flowrate for mobile phase. Chromatographic separations were performed by a UNISON UK-C18 250 × 4.6 mm column (Imtakt Co., OR, USA). Sodium formate (98%), sodium fumarate (99%), sodium succinate (98%), and sodium acetate (99%) were obtained from Junsei Chemical (Tokyo, Japan), TCI (Tokyo, Japan), Duksan Chemical Co., Ltd (Gyenggi-do, korea) and Sigma–Aldrich (St. Louis, MO, USA) for laboratory analysis, respectively.

3. Results and discussion

3.1 Phase I test (Biostimulation test)

To create anaerobic conditions and to stimulate indigenous dechlorinating micro-organisms, Phase I test (Biostimulation test) was performed at two monitoring wells (SKW-5 to SKW-4) by injecting formate and bromide during the first 15 d of the test.

In the last four years (2010–2013), the average annual rainfall at the test area was observed to be 1,632 mm, and approximately 75% of the annual

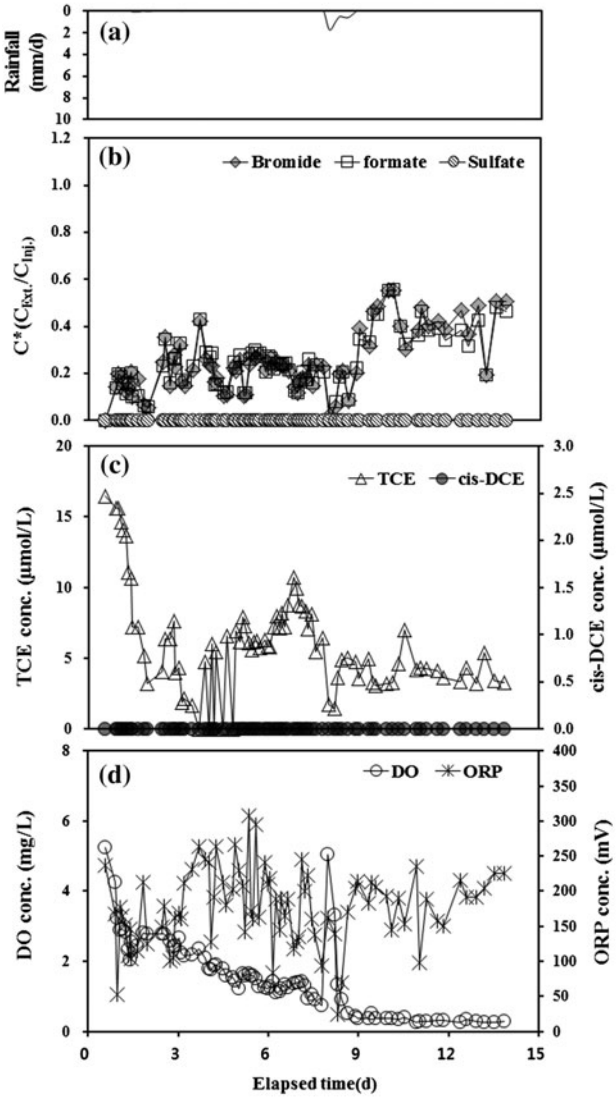

Fig. 2. Rainfall intensities at a test site (a), normalized concentrations of bromide, formate and sulfate (b) variation of TCE, its dechlorination product concentration, (c) and variation of DO and ORP concentration at a downgradient SKW-5 well during a test of phase I (d).

precipitation occurred during the rainy season from June to September. Phase I test was conducted during the dry season of April, 2012 and total precipitation during the period of Phase I test was 5 mm (Fig. 2(a) and Table 2).

Breakthrough curves for formate, bromide, and sulfate were plotted using relative concentrations, C^*, of the solutes (Fig. 2(b)). The relative concentration, C^*, was calculated by dividing the solute concentrations extracted from the SKW-4 (C_{ext}) by the solute concentrations injected into the SKW-5 (C_{inj}). If degradation of the solutes does not occur in the aquifer, C^* for formate, bromide, and sulfate will show similar patterns [29–32].

During a period of Phase I test, similar changes of C^* for formate and bromide were observed, indicating no formate removal through biodegradation by indigenous micro-organisms and no sorption onto aquifer solids at the test aquifer. These results can be confirmed by calculating the average ratio between C^* for formate and $C_{Br}{}^*$ for bromide (Table 2). That is, if the ratio is one, no removal of formate will have occurred, and if less than one, consumption of formate had occurred. During the period of Phase I, the average ratio with a 95% C.I. was 1.01 ± 0.03 (Table 2). Consequently, there was little removal of formate through biological, physical and chemical reactions.

A typical byproduct of TCE reductive dechlorination, cis-DCE was not detected during the Phase I test, although TCE concentrations apparently decreased (Fig. 2(c)). During the same period, ORP was kept nearly constant at 177 mV with a 95% C.I. of 12 (Table 2), and DO concentration decreased from 5.6 to 0.52 mg/L (Fig. 2(d)).

In general, strictly anaerobic conditions are required for the growth and control of denchlorinating microbes, since they are highly sensitive to oxygen [7,16,20]. Several studies reported that geochemical conditions such as DO, ORP, and other EAs are essential for an effective ARD process, and that ARD needs more reduced redox conditions [33–35]. Although the DO concentration decreased to the lower levels, ORP was kept much higher than the ranges of ORP where denitrification, sulfate reduction and methanogenesis generally occur. Therefore, no production of c-DCE, high ORP, and no biodegradation of formate indicate that the biological TCE degradation did not contribute to the decrease in TCE and this may be due to the variation in background TCE concentration.

3.2. *Phase II test (sulfate injection test)*

After 15 d of elapsed time, sulfate was simultaneously injected with formate and bromide with an

Table 2

Concentrations of solvents, solutes and DO and ORP at an extraction well of SKW-5 (average ± 95% confidence interval) during three sequential phases of well-to-well tests

Elapsed time (d)		Total rainfall (mm)	Average TCE conc. (μmol/L)	Average cis-DCE conc. (μmol/L)	[a]Normalized formate conc. ($C^*/C_{Br}{}^*$)	Normalized sulfate conc. ($C^*/C_{Br}{}^*$)	Normalized fumarate conc. ($C^*/C_{Br}{}^*$)	Average succinate conc. (mmol/L)	Average acetate conc. (mmol/L)	Average ORP (mV)	Average DO (mg/L)
Phase I	0–15	5	6.0 ± 0.77	[b]N.D.	1.01 ± 0.03	N.D.	N.D.	N.D.	N.D.	177 ± 12	1.6 ± 0.2
Phase II	35–45	10	1.6 ± 0.21	N.D.	0.68 ± 0.04	0.32 ± 0.06	N.D.	N.D.	N.D.	74 ± 20	0.16 ± 0.0003
Phase III	50–65	0	0.52 ± 0.06	0.71 ± 0.12	0.47 ± 0.12	0.52 ± 0.09	0.04 ± 0.03	0.50 ± 0.19	0.03 ± 0.02	−102 ± 27	0.16 ± 0.03
	65–90	430	0.68 ± 0.19	0.23 ± 0.10	0.30 ± 0.13	0.51 ± 0.14	0.08 ± 0.15	0.07 ± 0.03	0.21 ± 0.06	−22 ± 18	0.35 ± 0.10
	91–100	0	0.72 ± 0.06	0.41 ± 0.04	0.02 ± 0.002	0.34 ± 0.04	0.11 ± 0.002	0.11 ± 0.06	0.40 ± 0.06	−40 ± 15	0.19 ± 0.0003

[a] Normalized concentration of solute ($C^* = C_{ext}/C_{inj}$) is divided by Br-normalized concentration. (C_{ext}/C_{inj}).

[b] Not detected.

average sulfate concentration and a 95% C.I. of 0.68 ± 0.07 mmol/L to reduce the range of groundwater ORP, where TCE dechlorination could occur. It was decided that injection concentration of sulfate was not problematic for TCE denitrification. The proposed range of the initial sulfate concentration for effective and complete dechlorination of TCE was 2.6–3.1 mmol/L [36]. Phase II was conducted during the dry season from May to June, 2012 and total precipitation for 30 d was 72 mm (Fig. 3(a)). Thus, oxygenated groundwater effects on the TCE dechlorination would be minimal.

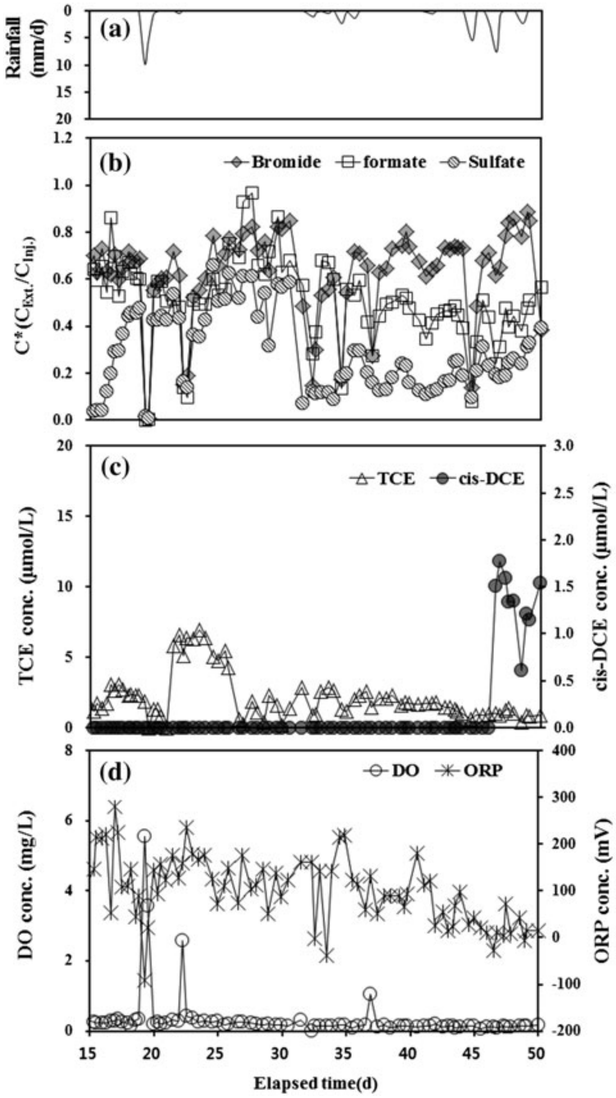

Fig. 3. Rainfall intensities at a test site (a), normalized concentrations of bromide, formate and sulfate (b) variation of TCE, its dechlorination product concentration (c), and variation of DO and ORP concentration at a downgradient SKW-5 well during a test of Phase II (d).

During the first 15 d of phase II (i.e. 15–35 d of elapsed time), similar changes in *C** for formate, bromide, and sulfate were observed (Fig. 3(b)). However, after 35 d of elapsed time, *C** values for sulfate and formate started to become lower than those of bromide. The ratios of C^*/C_{Br}^* for formate and sulfate were 0.68 ± 0.04 and 0.32 ± 0.06, respectively (Table 2). These results suggested that approximately 32% of formate and 68% of sulfate were possibly removed through biological, chemical, and/or physical processes.

cis-DCE, a typical byproduct of TCE reductive dechlorination, was detected at the end of the test. (Fig. 3(c)). Other byproducts such as 1,1-dichloroethen (1,1-DCE), trans-1,2-dichloroethen (trans-DCE), VC and ETH were not detected (data not shown). At that time, TCE concentrations were decreasing, while cis-DCE concentrations increased and a final ED for dechlorination, dissolved H_2, started to show up in the readings, ranging from 100 to 200 nmol/L (data not shown). Simultaneous detection of cis-DCE and H_2 suggest that biological reductive dechlorination might have occurred.

ORP was continuously decreased from 170 to 0 mv (Fig. 3(d)) by an average, and 95% C.I. of 74 ± 20 mV (Table 2), and the average concentration of DO was 0.16 mg/L with a 95% C.I of 0.0003 (Table 2). The decrease in both ORP and DO concentration and the consumption of sulfate suggest that the tested aquifer had changed to a more reduced condition in comparison to the previous Phase I test.

Dechlorination of TCE prefers more reduced conditions such as iron reducing, sulfate-reducing and methanogenic conditions [9,37,38]. Also, El Mamouni et al. [36] reported that reductive dechlorination of TCE to cis-DCE was not inhibited by high sulfate concentrations of 10–15 mmol/L. At two different sulfate concentrations of 10–15 mmol/L, degradation efficiency of TCE to cis-DCE were 54–81%, respectively. In other studies sulfate reduction occurred simultaneously without inhibition of TCE dechlorination, when ED was constantly fed [39–42]. Decrease in ORP and TCE dechlorination to cis-DCE might be triggered by sulfate injection.

3.3. Phase III test (Activity test under high recharge)

Phase III was conducted during the rainy season from June to July, 2012 and the total precipitation for 25 d was 430 mm (Fig. 4(a)). The precipitation was approximately 36% of that year's precipitation in this area. Thus, oxygenated groundwater effects on the TCE dechlorination would be greater than those of Phase I and II tests. Phase III tests were carried out to

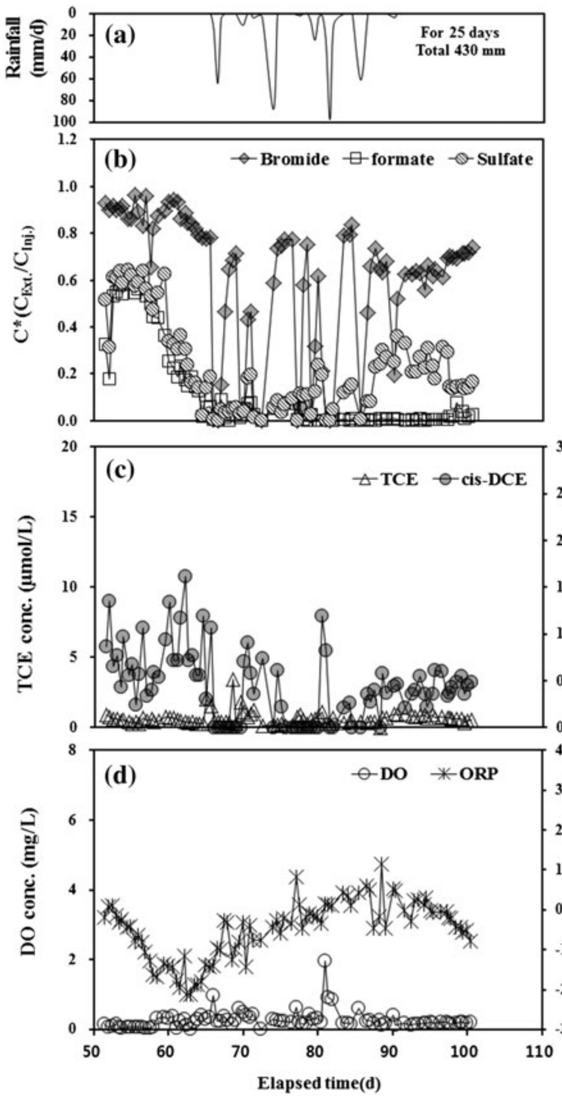

Fig. 4. Rainfall intensities at a test site (a), normalized concentrations of bromide, formate and sulfate (b) variation of TCE, its dechlorination product concentration (c) and variation of DO and ORP concentration at a downgradient SKW-5 well during a test period of Phase III (430 mm rainy season) (d).

evaluate the effects of heavy precipitation on the dechlorination activity.

During the 50–65 d of elapsed time, there was no precipitation. Consumption activity of formate and sulfate was initially stable (Fig. 4(b)). After 56 d of elapsed time, significant consumption of both formate and sulfate was observed. Also, greater TCE degradation activity resulted in higher cis-DCE concentrations (Fig. 4(c)), lower level of DO and ORP than those of Phase I and II (Fig. 4(d)) were observed, indicating that more active and stable dechlorinating populations were possibly stimulated in the tested aquifer.

During the 65–90 d of elapsed time, 430 mm of heavy precipitation for 25 d most likely resulted in the flowing of high oxygenated groundwater into the active dechlorination zone. High recharge was indicated by the fluctuation of C^* for bromide. C^* for bromide dropped to nearly zero just after heavy rainfall recharge. For example, after approximately 64.5 mm/d precipitation at the elapsed time of 65 d, C^* for bromide dropped from 0.78 to 0.01.

This phenomena might be due to the geological properties. Other studies reported that the tested aquifer was composed of gravelly sand with high permeability. The test bed aquifer also has a high recharge factor because the study location was surrounded by a sloped forest [26,28]. This high rainfall recharge was also assured by the increase in ORP during the same period (Fig. 4(d)). However, DO was mostly not affected by the rainfall recharge (Fig. 4(d)). It is plausible that DO might be consumed by the aerobic respiration of formate.

During the period of heavy rainfall (65–90 d of elapsed time), continuous detection of cis-DCE indicated that TCE dechlorination activity was maintained (Fig. 4(c)). However, the average cis-DCE concentrations decreased by a factor of 0.7 in comparison to those before the heavy rainfall (Table 2). Thus, the heavy rainfall recharge partially deteriorated the TCE dechlorination activity.

After the heavy rainfall (90–100 d of elapsed time), it is important that consumption of solutes injected and production of cis-DCE recovers. Comparing the average C^*/C_{Br}^* values for formate and sulfate before and after heavy rainfall recharge showed that the ratios for formate decreased from 0.47 ± 0.12 to 0.02 ± 0.002 and that the values for sulfate also decreased from 0.52 ± 0.09 to 0.34 ± 0.04 (Table 2). These results indicate a 45 and 18% increase of formate and sulfate consumption, respectively.

Consumption of TCE and production of cis-DCE was also observed after the heavy rainfall. This result suggests that the process was stable and robust. Most of the dechlorinating microbes grew on the surface of aquifer solids by forming biofilms, so widespread ED through liquid/nitrogen gas spray injection had protected and reduced microbes' exposure to oxygen. In addition *tce*A reductase degrading TCE to cis-DCE was less sensitive to oxygen than other dechlorination reductase such as VC reductase (*bvc*A and *vcr*A) [23]. Therefore the application of reductive dechlorination is possible at highly recharged aquifer with consistently injected formate as an ED. But other dechlorinated compounds such as VC and ETH were not detected even though EDs were continuously injected for a year (data not shown). It would be of interest if the partial dechlorination of TCE was due to the lack of complete dechlorinating micro-organisms or inactivation of c-DCE and VC reductase by high recharge of oxygenated water.

3.4. Fumarate as an indicator of biological TCE dechlorination activity

Several studies reported that fumarate could be an alternate EA in identifying correlations with biological reductive dechlorination PCE and/or TCE. Fumarate is biologically reduced and fermented to succinate and acetate by dechlorinating microbes as a growth substrate and an alternative EA [43–48]. Therefore, fumarate was adapted to confirm the production of cis-DCE from TCE. Fumarate was co-injected with the solutes including formate, sulfate, and bromide during Phase III.

Without the period of acclimation, fumarate consumption was observed (Fig. 5(b)), and its average values for C^*/C_{Br}^* was less than 0.11 ± 0.002 during the whole period of the test. The immediate degradation of fumarate suggests that microbes stimulated during the Phase I and II tests were directly involved in the degradation of fumarate. A biological reduction product of fumarate, succinate was detected and its concentration increased just after 2 d of injecting fumarate (Fig. 5(c)), In addition, acetate, a fermentation product of fumarate, was detected after 15 d of injecting fumarate. The detection of succinate and acetate would be an evidence of biological degradation of fumarate by the microbes stimulated in the Phase I and II tests.

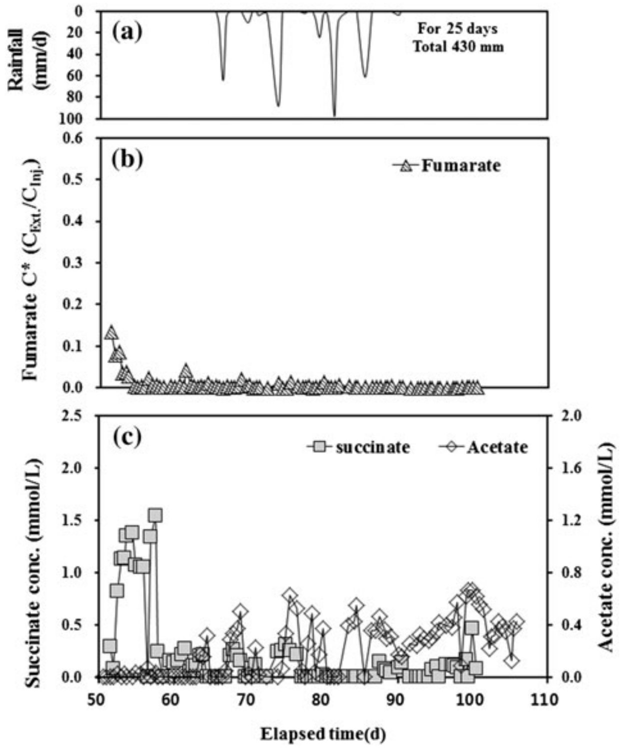

Fig. 5. Rainfall intensities at a test site (a), normalized concentrations of fumarate (b) variation anaerobically biological product of fumarate (succinate and acetate) concentration (c) at a downgradient SKW-5 well during a test of Phase III (430 mm rainy season).

Some dechlorinating micro-organisms have an ability to carry out both fumarate respiration and TCE reductive dechlorination [47,48]. *Desulfitobacterium dehalogenans* can also use fumarate as EA with formate and pyruvate as EDs [45]. *Desulfuromonas michiganesis* sp. nov. reductively dechlorinated PCE to cis-DCE grew best in the presence of fumarate [43]. Hageman et al. [49] reported that transformation of fumarate was highly correlated with reductive dechlorination. In this study immediate fumarate reduction to succinate would be an evidence of biological TCE reductive dechlorination to cis-DCE.

4. Conclusions

In this study, a well-to-well test consisting of three sequential phases was carried out to investigate the variation in reductive TCE dechlorinaton activity under oxygenated groundwater recharged conditions. In Phase I, there was not biological activity of TCE by injecting only formate as an ED. After injecting both formate and sulfate during Phase II, the tested aquifer was changed to a more reduced condition, and biological reductive dechlorination of TCE occurred. In the dry period of Phase III, during which fumarate was provided as an additional ED, both the consumption of formate and sulfate and the dechlorination activity of TCE to cis-DCE were enhanced relative to those observed in Phase II. Over the higher recharge period (25 d) of Phase III, there was heavy precipitation in 430 mm to account for 35% of the annual precipitation. Although oxygenated water was substantially recharged into the test aquifer, the consumption of formate and sulfate was continuously observed.

On the other hand, the production of cis-DCE via TCE dechlorination was decreased to 70% of that occurred before the recharged period (0.71–0.23 μmol/L). Following the cessation of heavy rainfall, however, the dechlorination activity of TCE was recovered as evidenced by the average cis-DCE concentration increasing from 0.23 to 0.41 μmol/L. The sustained dechlorination activity was accompanied by fumarate degradation to its anaerobically biological products such as succinate and acetate. This finding suggests that the bio-active zone generated by continuously injection of EDs and sulfate at the test aquifer is stable and robust. Further studies are needed to confirm the subsequent dechlorinating activities of the TCE degradation products (e.g. DCE isomers and VC) during the high recharge season.

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