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A novel three-stage treatment train for the remediation of trichloroethylene-contaminated groundwater

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Abstract

This study used a novel three-stage treatment train that was composed of chemical oxidation, anaerobic bioremediation and passive reactive barrier to remediate trichloroethylene (TCE)-contaminated groundwater. Batch oxidation and biodegradation experiments and a continuous column study were used to evaluate the compatibility of different technologies and the feasibility of the removal of TCE by the treatment train. The results of batch experiments show that high concentrations of TCE (50 mg L\(^{-1}\)) were removed completely by the addition of 5,000 to 50,000 mg L\(^{-1}\) persulfate during 24 to 96 h of reaction. Ferrous ion-activated persulfate may result in a residue of TCE due to the rapid consumption of persulfate by ferrous ions. Significant inhibition of soil bacteria was observed upon the addition of persulfate in concentrations greater than 20,000 mg L\(^{-1}\). Both low pH and the oxidative stress of persulfate were responsible for the adverse effect on indigenous microorganisms. The results of a microcosm study reveal that the presence of high concentrations of sulfate (up to 50,000 mg L\(^{-1}\)) had no adverse effect on TCE removal. Sulfate significantly enhanced the dechlorination of vinyl chloride via sulfate reduction, which demonstrates that sulfate produced from persulfate oxidation could be utilized
by indigenous bacteria to achieve the complete dechlorination of TCE. The addition
of 5,000 to 50,000 mg L\(^{-1}\) bioremediation reagent improved the degradation of TCE.
Dechlorinating bacteria, *Dehalococcoides*, and the reductive dechlogenase, *vcrA*, of
*Dehalococcoides* were detected during TCE biodegradation. The results of a column
study show that the proposed treatment train removed TCE and its byproducts
effectively and there was no problem with the connection of chemical oxidation and
anaerobic bioremediation in the novel treatment train technology. The use of 10,000
mg L\(^{-1}\) of persulfate and the bioremediation reagent, and the PRB that can
continuously release 6,000 mg L\(^{-1}\) of persulfate are suggested to operate the treatment
train. The proposed treatment scheme will provide a more effective alternative for the
remediation of contaminated sites in the future.

**Keywords:** Trichloroethylene (TCE); treatment train; persulfate; anaerobic
bioremediation; passive reactive barrier (PRB); persulfate-releasing material
1. Introduction

Soil and groundwater contamination is ubiquitous and usually difficult to treat because of complex geological, biological, hydraulic and pollution conditions in sites.

Treatment trains are used when no single technology is capable of treating all of the contaminants in a particular medium. Two or more innovative and established technologies can be used together in treatment trains, which are either integrated processes or a series of treatments that are combined in sequence to provide the necessary treatment. Many technologies, such as bioremediation, electrochemical treatment, photocatalytic oxidation, chemical oxidation/reduction, permeable reactive barriers and ultrasonication, have been used for treatment trains to remediate contamination in soil and groundwater. The synergy can achieve results that are better than the sum of the effect of the individual technologies.

Trichloroethylene (TCE) is widely used as a cleaning agent for industrial metals, metal degreasing and dry cleaning operations and is one of the common contaminants that is observed in soil and groundwater. Of the various treatment methods, in situ chemical oxidation (ISCO) is a widely used remediation technology for the in-situ treatment of contaminated soils and groundwater. Common ISCO oxidants that are
used for the remediation of contaminated sites include hydrogen peroxide (H₂O₂),
such as Fenton’s reagent and Fenton-like reaction, ozone (O₃), and permanganate
(MnO₄⁻). Persulfate (PS) (S₂O₈²⁻) is a newer oxidant that can be activated to
promote the formation of sulfate free radicals (SO₄⁻⋅), which are instrumental in the
destruction of chlorinated solvents such as chlorinated ethanes and chlorinated
ethenes. Persulfate is a strong oxidant with a high redox potential of 2.01 V for
the half-cell reaction that is shown below:

\[ S_2O_8^{2-} + 2e^- \rightarrow 2SO_4^{2-}, \quad E^0 = 2.01 \text{ V} \]  

Persulfate can be activated by various activators to form more powerful sulfate free
radicals (SO₄⁻⋅), which have a higher redox potential of 2.60 V. When heat or UV
light is applied, one mole of persulfate produces two moles of sulfate free radicals, as
shown in reaction (2):

\[ S_2O_8^{2-} + \text{heat or } hv \rightarrow 2SO_4^{-\cdot} \]  

With the activation of transition metals (represented by M), one mole of persulfate
produces one mole of sulfate free radicals:

\[ S_2O_8^{2-} + M \rightarrow M^+ + SO_4^{2-} + SO_4^{-\cdot} \]  

The half-cell reaction for sulfate free radicals is:
$$SO_4^- \cdot +e^- \rightarrow SO_4^{2-}, \quad E^0 = 2.60 \, \text{V}$$  \hspace{1cm} (4)

Persulfate oxidation is used to degrade various contaminants, such as chlorinated organic compounds and petroleum hydrocarbons\textsuperscript{20, 21}. Recently, persulfate-releasing materials that are composed of persulfate, cement and sand have been successfully developed to remediate groundwater that is contaminated. The persulfate-releasing materials can form an ISCO barrier system that releases persulfate for a long period of time, which results in continuous degradation of contaminants in the subsurface\textsuperscript{22, 23}.

Although ISCO treatment is effective for the remediation of contaminated sites, the cost of ISCO may be high due to the expense of the oxidant. In addition, when the concentrations of the contaminants are low, most of the oxidants added to the subsurface may be substantially consumed by natural organic matter. Consequently, to ensure better remediation, treatment trains such as chemical oxidation coupled with bioremediation have been proposed\textsuperscript{24}. Bioremediation is an environmentally friendly and cost-effective remedial technology. However, TCE is not biodegraded by direct metabolism under aerobic conditions. Although TCE can be removed via aerobic co-metabolism, TCE biodegradation appears to be more effective under anaerobic conditions\textsuperscript{25, 26}. In situ bacteria, including nitrate-, iron- and sulfate-reducing bacteria
and methanogens remove TCE via reductive dechlorination under anaerobic conditions \(^{27-29}\). It has been reported that the dechlorination of TCE is enhanced by sulfate-reducing bacteria under anaerobic conditions \(^{29, 30}\). Koenigsberg \(^{31}\) indicated that sulfate-reducing conditions are the predominant microbiological conditions during the enhanced biological treatment of chlorinated hydrocarbon-contaminated groundwater using Hydrogen Release Compound (HRC\(^{TM}\)). It is noteworthy that although the degradation of TCE can be achieved under anaerobic conditions, dichloroethene (DCE) and more toxic vinyl chloride (VC) may be produced and can accumulate during the reductive dechlorination of TCE \(^{32}\).

As shown in Reaction (1), two moles of sulfate are produced when one mole of persulfate is consumed. Since sulfate is an electron acceptor for anaerobic biodegradation, it may be feasible to use persulfate followed by anaerobic bioremediation to enhance the removal of TCE in the subsurface. However, before the combined remedial scheme is applied, it is necessary to evaluate the effects of sulfate on the biodegradation of TCE and the compatibility of chemical oxidation and anaerobic bioremediation. The accumulation of DCE and VC during anaerobic dechlorination of TCE must also be considered.
While high oxidant concentrations (10-30% wt/wt) are usually used in field application\textsuperscript{33, 34}, this study attempts to apply lower oxidant concentrations coupled with an anaerobic biodegradation to treat contaminated groundwater. A three-stage treatment train that was composed of persulfate oxidation, anaerobic bioremediation and passive reactive barrier (PRB) was developed to remediate TCE-contaminated groundwater. The main objectives of this study were to: (1) evaluate the efficiency of persulfate oxidation and anaerobic bioremediation in TCE removal; (2) assess the effect of sulfate concentrations on the anaerobic biodegradation of TCE; (3) evaluate the feasibility of combining persulfate oxidation, anaerobic bioremediation and persulfate-releasing materials for the remediation of a TCE-contaminated aquifer; (4) determine the optimal operational conditions for the three-stage treatment train system and (5) evaluate the effects of the proposed treatment train on indigenous microbes.

2. Materials and Methods

2.1 Chemicals and materials

The chemicals that were used for this study are: TCE (99.9%, J. T. Baker, USA), cis-DCE (>99%, Tokyo Chemistry Industrial Co., Japan), trans-DCE (>98%, Tokyo
Chemistry Industrial Co., Japan), 1,1-DCE (> 99%, Merck, USA), VC (2,000 ppm in methanol, Supelco, USA) and sodium persulfate (> 99%, Riedel-de Haen, Germany).

An anaerobic bioremediation reagent, EcoClean™, which is composed of hydrocarbons and amino acids, was purchased from Ecocycle Co., Japan. Persulfate-releasing materials that release oxidant continuously were synthesized similarly to the method of a previous study by the authors. The mass ratio of persulfate/cement/sand/water for the persulfate-releasing materials was 1/1.4/0.24/0.7. Aquifer sediments and groundwater samples used for the experiments were collected from a TCE-contaminated site in southern Taiwan. All of the samples were stored at 4°C before use. The values for pH, moisture content, total organic carbon (TOC), cation exchange capacity (CEC), oxidation-reduction potential (ORP) and the total bacteria of the soil were 7.54, 16%, 2.56%, 8.19 meq/100 g, 98 mV and $1.3 \times 10^5$ CFU g$^{-1}$ soil, respectively. A mineral medium that contained buffer solution, calcium and magnesium solutions, and trace elements was used for anaerobic biodegradation experiments.
2.2 Batch oxidation experiments

Batch oxidation experiments were conducted using 60 mL serum bottles for different persulfate concentrations and PS/Fe(II) ratios, in order to evaluate the appropriate conditions for TCE oxidation. Each bottle was filled with 10 g of the aquifer soil and 50 mL DI water with the required TCE, persulfate, and ferrous ion concentrations. A set without persulfate addition was constructed to evaluate the possible biodegradation of TCE during the oxidation experiments. Control bottles containing 250 mg L$^{-1}$ of HgCl$_2$ were autoclaved twice before use. All batch experiments were conducted in duplicate and kept at 25 ± 2°C in darkness, until analysis. The detailed components of the batch oxidation experiments are shown in Table 1.

2.3 Batch anaerobic biodegradation experiments

A microcosm study was conducted using 60 mL serum bottles, in order to assess the ability of intrinsic bacteria to dechlorinate TCE and to determine the effect of EcoClean and sulfate concentration on TCE biodegradation under anaerobic conditions. The effect of sulfate concentration on TCE removal in the presence of
EcoClean was also determined. Microcosms contained 10 g of the aquifer soil as the sources of microorganisms and 50 mL mineral nutrients with the required TCE, EcoClean, and sulfate concentrations. Aquifer soil and the mineral nutrients were purged with \( \text{N}_2 \) to eliminate \( \text{O}_2 \) before use. The headspace of each serum bottle was filled with \( \text{N}_2 \) to keep the microcosm anoxic. Control bottles containing 250 mg L\(^{-1}\) of \( \text{HgCl}_2 \), and inocula for the control groups, were autoclaved twice before use. pH in all microcosms was around 6.5-7 during the experiments. All of the microcosm studies were conducted in duplicate and kept at 25 ± 2\(^{\circ}\)C in darkness, until analysis. The detailed components of the microcosms are shown in Table 1.

2.4 Column study

A column study was performed to evaluate the ability of the proposed three-stage treatment train to remediate TCE-contaminated groundwater. The treatment train that was used in this study consisted of persulfate oxidation, anaerobic bioremediation reagent, and a PRB (persulfate-releasing materials). A total of five continuous-flow glass columns were used to simulate the treatment train system. Figure 1 shows the layout of the column experiments. Five columns were used in sequence; i.e.,
persulfate oxidation for the first column, anaerobic bioremediation for the second and
the third columns, PRB treatment for the fourth column and the fifth column was used
for monitoring. Each glass column was 30-cm long, with an inner diameter of 5 cm.
All columns were filled with in situ aquifer sediment that was sampled from a
TCE-contaminated site in southern Taiwan, except for Column 4. In situ groundwater
with 50 mg L$^{-1}$ of TCE (spiked) and 10,000 mg L$^{-1}$ of persulfate solution were stored
in two gas-sampling bags, in order to prevent the TCE from becoming volatile and to
prevent water evaporation during the experiment. TCE and persulfate solutions were
continuously pumped into the columns by a peristaltic pump. The flow rate and
average pore volume for the column study were 0.24 mL/min and 178 mL,
respectively.

Column 1 simulated the first stage of the treatment train system. It was expected
that high concentrations of TCE would decrease rapidly and significantly in Column 1.
The result of a pre-test showed that the output TCE concentration for Column 1 was 5
mg L$^{-1}$, after the injection of persulfate. Therefore, Columns 2 and 3 were pre-filled
with 5 mg L$^{-1}$ TCE contaminated soil, followed by a concentration of 10,000 mg L$^{-1}$
of EcoClean reagent, in order to evaluate the effectiveness of EcoClean on the
anaerobic reductive dechlorination of low concentrations of TCE from Column 1. In Column 4, a PRB that contained 480 g persulfate-releasing materials which can continuously release persulfate with an average concentration of 6,000 mg L\(^{-1}\) was constructed to oxidize the residual TCE, DCEs, and VC from Columns 2 and 3. Column 5 represented the down-gradient area of the remedial system.

2.5 Microbial analysis

A total heterotrophic count was performed to determine the effect of persulfate and EcoClean on intrinsic bacteria, during all experiments. A Difco\textsuperscript{TM} plate count agar (Becton, Dickinson and Co., USA) was used to assess the total number of bacteria in sediment samples, using the spread plate method\textsuperscript{36}. During the batch anaerobic biodegradation experiments, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to detect the variation in the bacterial community. DNA was extracted from 0.5 g soil samples using a PowerSoil\textsuperscript{®} DNA Isolation Kit (Mo Biol, USA). The V6-V8 region of the 16S rDNA was amplified using the primers, 968F (5\textquoteleft-AAC GCG AAG AAC CTT AC-3\textquoteleft) and 1401R (5\textquoteleft-CGG TGT GTA CAA GAC CC-3\textquoteleft). PCR amplification used an initial denaturation at 97 °C.
for 5 min and then 28 cycles of annealing at 95 °C for 1 min, 54 °C for 40 sec and 72
°C for 30 sec, followed by a final extension at 72 °C for 7 min. Each amplified PCR
product underwent DGGE using a Bio-Rad DCode system (Bio-Rad, Hercules, CA,
USA), in order to monitor the changes in the microbial diversity during the
experiments. A 10% polyacrylamide gel with a 40-60% denaturant gradient
underwent electrophoresis at 60 °C and 65 V for 870 min. After electrophoresis, the
gels were stained using the silver-stain method. Quantity One 4.6.8 software (BioRad,
USA) was used to analyze DGGE banding patterns. Dendrograms were created using
the algorithm for the unweighted pair-group method using arithmetic averages
(UPGMA) for cluster analysis. Real-time (RT)-PCR (quantitative PCR, qPCR) was
performed using a LightCycler® 480 (Roche, Germany) to detect the expression of
Dehalococcoides (968F: ACG TGC CAG CAG CCG CGG TA; 1401R: TCC TCC
CCG TTT CGC GGG GCA) and the reductive dechlogenase, \( vcrA \), of
Dehalococcoides (968F: TGC TGG TGG CGT TGG TGC TCT; 1401R: TGC CCG
TCA AAA GTG GTA AAG). All of parameters for these procedures, including the
annealing and polymerization temperatures, the primer concentrations and the MgCl_2
concentration for qPCR, followed the recommendations given in the procedures of the
2.6 Analytical methods

Aqueous samples were pretreated using a purge and trap equipment and then analyzed for TCE, cis-DCE, trans-DCE, 1,1-DCE and VC using a gas chromatography (7890A, Agilent Technologies, USA) equipped with a flame ionization detector and capillary column (GsBP-624, 60 m × 0.32 mm). The operating temperatures were maintained at 180°C for the injector and 230°C for the detector. The oven temperature was initially maintained at 35°C for 5 min, then elevated at a rate of of 11°C min\(^{-1}\) to 115°C, and held at 115°C for 3 min. The temperature was then raised at a rate of 20°C min\(^{-1}\) to 220°C, and then maintained at 220°C for 1 min.

Sulfide and persulfate were respectively analyzed using a spectrophotometer (DR 5000, Hach Company, USA) according to Methylene-Blue method \(^{36}\) and the method of Huang et al. \(^{16}\). Dissolved organic carbon (DOC) was analyzed using a total carbon analyzer (Phoenix 8000, Tekmar Dohrmann, USA) \(^{36}\). The pH and redox potential values were measured using a pH meter (SUNTEX SP-2200, Taiwan) and an ORP meter (ORION Model 250A+, Thermo Fisher Scientific), respectively.
3. Results and discussion

3.1 The effect of persulfate and ferrous ion concentrations on TCE degradation and the number of soil bacteria

Figure 2a shows that during 24 to 96 h of reaction, 100% of the TCE was removed using different persulfate dosages. The results of the experiment without persulfate addition show that TCE was not biodegraded during 96 h of incubation. Therefore, in situ microorganisms did not contribute to the removal of TCE during the oxidation experiments. The DO concentrations in the bottles were around 5.7 mg L$^{-1}$ during the experiment. Although TCE may be biodegraded via cometabolic biodegradation using soil organic matter as the carbon source under aerobic conditions$^{37}$, aerobic TCE biodegradation was not observed in this study. Longer incubation time may be needed to evaluate the potential of intrinsic TCE biodegradation under aerobic conditions.

The pseudo-first order rate constants were $9.05 \times 10^{-2}$, $1.89 \times 10^{-1}$, $3.39 \times 10^{-1}$ and $3.73 \times 10^{-1}$ h$^{-1}$ for the addition of 5,000, 10,000, 20,000 and 50,000 mg L$^{-1}$ persulfate, respectively. The TCE degradation rate increased as the amount of persulfate that was added increased. Liang et al.$^{38}$ and Fang et al.$^{39}$ found that sulfate radicals were
predominant under acid and neutral conditions at ambient temperature (10-30 °C). In this study, the temperature and pH in the persulfate systems were 25°C and 4 to 6.5, respectively. This indicates that sulfate radicals mainly contributed the removal of TCE. In addition, sulfate radicals can react with water to produce hydroxyl radicals under all pH conditions, as shown in reaction (5):\[ SO_4^- + H_2O \rightarrow \cdot OH^- + SO_4^{2-} + H^+ \] (5)

Since hydroxyl radicals preferred to attack unsaturated double bond in TCE, the produced hydroxyl radicals may also play a role in the degradation of TCE. It should be noted that although it is not difficult to treat TCE in the bench-scale systems, the rebound of contaminants was most prevalent at chlorinated solvent-contaminated sites applying chemical oxidation. Therefore, more efforts (e.g., treatment trains) need to be implemented to prevent rebound occurrence in the field. Figure 2b shows that more than 70% of persulfate remained in the system at the end of the experiments, which demonstrated that persulfate was quite persistent. Since the applied persulfate concentrations removed TCE effectively during a short time of reaction, a study using persulfate less than 5,000 mg L\(^{-1}\) could be performed in the future to make a balance between cost and time. The results show that persulfate
treatment rapidly decreased high concentrations of TCE so it is a suitable first stage in
the treatment train.

The total number of soil bacteria changed from an original value of $1.3 \times 10^5$ to
$1.4 \times 10^5$, $2.6 \times 10^4$, $1.3 \times 10^3$, $1.3 \times 10^2$ and $6.8 \times 10^1$ CFU g$^{-1}$ soil upon the respective
addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L$^{-1}$ persulfate, at 96 h reaction
time. The inhibition of soil bacteria increased as the amount of persulfate that was
added increased. As shown in Figure 2c, after the addition of persulfate, especially for
the 20,000 and 50,000 mg L$^{-1}$ persulfate batches, the pH decreased from 7 to 5.1 and
4.3, respectively, which was the main reason for the inhibition of the total soil
bacteria. In general, the persulfate reaction results in a pH decrease in soil and/or
aquifers that have a low buffering capacity. Decreases ranging from 2.5 to 6 pH units
have been observed under laboratory conditions, for both aqueous $^8, ^{20}$ and soil slurry
experiments $^{42}$. Tsitonaki et al.$^{42}$ and Richardson et al.$^{43}$ found that significant
decreases in microbial density were observed after exposure to persulfate due to the
drop of pH. Our findings are consistent with the above studies. It should be noted that
although the pH was about 6.5 in the experiments where 5,000 and 10,000 mg L$^{-1}$
persulfate was added, the number of bacteria also decreased. This phenomenon
demonstrates that in addition to pH, the oxidative stress of persulfate also has an adverse effect on indigenous microorganisms. Sutton et al.\textsuperscript{33} also indicated that oxidative stress from chemical oxidation had an effect on soil bacteria. The decrease in the total number of soil bacteria may be due to the presence of sulfate radicals in the system. In addition, persulfate alone may also kill bacteria directly. Dogan-Subasi et al.\textsuperscript{44} evaluated the effect persulfate on microbial dechlorination activity under different persulfate concentrations (0.01-4.52 g/L). No gene expression and cell activity were detected with the addition of 1.13-4.25 g/L persulfate. Since the decrease in TCE concentration was not observed under these persulfate concentrations, the author concluded that persulfate was not activated in the experiments. It has been demonstrated that the oxidative stress caused by chlorine, permanganate, and hydrogen peroxide can inactivate bacteria\textsuperscript{45,46}. The redox potential of persulfate is 2.01 V, which is higher than that of chlorine (1.4 V), permanganate (1.7 V), and hydrogen peroxide (1.8 V)\textsuperscript{34}. Therefore, the oxidative stress caused by both persulfate and sulfate radicals contributed to the inhibition of bacterial growth in the experiments. Since adding persulfate at concentrations of 5,000 and 10,000 mg L\textsuperscript{-1} removed TCE without causing severe damage to in situ bacteria, persulfate
concentrations between 5,000 and 10,000 mg L\(^{-1}\) were selected for the treatment train in the following experiments.

Ferrous ions serve as electron donors that activate persulfate to generate sulfate radicals (see Eq. (3)). Therefore, the degradation of TCE by ferrous ion (Fe(II))-activated PS was studied, in order to better understand the effect of the PS/Fe(II) molar ratio on TCE removal. Figure 3a shows the efficiency with which TCE was removed by ferrous ion-activated persulfate. After 96 h of reaction time, TCE is completely removed by persulfate alone and using PS/Fe(II) molar ratios of 100/1 and 100/10. When PS/Fe(II) with molar ratio of 100/20 was added, only 80% of TCE was removed, which was a lower figure than that for the unactivated persulfate batch. Figure 3b shows that the ferrous ions in the PS/Fe(II) with molar ratio of 100/20 were totally consumed within 24 h of reaction, which resulted in the incomplete removal of TCE. The pseudo-first order rate constants were \(9.05 \times 10^{-2}\), \(48.6 \times 10^{-2}\), \(190.5 \times 10^{-2}\) and \(4.24 \times 10^{-2}\) h\(^{-1}\) for the addition of persulfate alone and PS/Fe(II) with molar ratios of 100/1, 100/10 and 100/20, respectively. The results show that although TCE removal was enhanced by ferrous ion-activated persulfate, there can be a residue of the contaminant because of the excess consumption of
persulfate by ferrous ions. Liang et al.\textsuperscript{8} used persulfate that was activated by ferrous ions to degrade TCE. Their results show that organic contaminants are more efficiently destroyed by sequentially adding controlled amounts of ferrous ions. However, if ferrous ion levels become excessive, it appears that ferrous ions scavenge sulfate free radicals, which results in a decrease in the efficiency with which contaminants are destroyed.\textsuperscript{8} Chen et al.\textsuperscript{47} also reported that excess addition of ferrous ion causes a decrease in the rate of degradation of methyl tert-butyl ether (MTBE) because there is competition for sulfate free radicals between ferrous ions and MTBE. Therefore, an appropriate dosage of ferrous ion needs to be selected when ferrous-ion activated persulfate is applied. Figure 3b also shows that large amounts of persulfate were consumed when ferrous ions were added. Although TCE is removed more efficiently when an appropriate amount of ferrous ions is added to the persulfate oxidation system, there is additional consumption of persulfate. Therefore, if the remediation time is allowed, the addition of ferrous ion may not be necessary, which would eliminate the need and cost of further persulfate injection.

As shown in Figure 3c, after PS/Fe(II) with molar ratios of 100/1, 100/10, and 100/20 were added, the pH decreased from 7 to 6.1, 6.1, and 5.9, respectively. The
final pH in the PS/Fe(II) batches was slightly lower than that in the persulfate alone
batch (pH 6.5) due to the addition of acid ferrous ions. The total soil bacteria number
decreased from $1.3 \times 10^5$ to $2.65 \times 10^4$, $2.78 \times 10^4$, $3.59 \times 10^4$ and $2.56 \times 10^4$ CFU g$^{-1}$ soil
for the addition of persulfate alone and PS/Fe(II) with molar ratios of 100/1, 100/10
and 100/20, respectively, at 96 h reaction time. These experimental results show that
both non-activated persulfate and ferrous iron-activated persulfate have an effect on
the total number of soil bacteria.

3.2 The effect of sulfate concentration on the anaerobic biodegradation of TCE and
the number of soil bacteria

Sulfate ($\text{SO}_4^{2-}$) is one of the persulfate oxidation products which may affect the
biodegradation of TCE and the survival of soil bacteria. Therefore, the effect of
sulfate on anaerobic TCE biodegradation was studied. Figure 4a shows that, after 100
d reaction time, 50, 55, 52, 53 and 50% of the TCE was biodegraded in the batches
containing 0, 5,000, 10,000, 20,000 and 50,000 mg L$^{-1}$ of sulfate, respectively. The
pseudo-first order rate constants for TCE degradation by in-situ bacteria were $7.4 \times$
$10^3$, $8.4 \times 10^3$, $8.3 \times 10^3$, $7.8 \times 10^3$ and $8.0 \times 10^3$ d$^{-1}$, respectively. The results
indicate that indigenous microorganisms at the contaminated site were capable of
degrading TCE. All of the batch experiments show that a similar amount of TCE was
removed. Therefore, the sulfate generated by persulfate oxidation process in the
treatment train technology has no effect on anaerobic TCE degradation by indigenous
bacteria.

Figures 4b to 4e show that the production of less-chlorinated byproducts,
including cis-DEC, trans-DCE, 1,1-DCE and VC occurred during TCE
biodegradation, which demonstrates that TCE is removed via anaerobic reductive
dechlorination. It should be noted that high concentrations of cis-DCE and VC
accumulated during the experiments and VC was not completely removed at the end
of the experiments when no sulfate was added. The presence of sulfate reduced the
accumulation of cis-DCE and VC and enhanced the dechlorination of VC effectively
during the anaerobic reductive dechlorination of TCE.

Figure 5a shows the production of sulfide in sulfate addition systems. Significant
sulfide concentrations ranging from 0.35 to 0.80 mg L\(^{-1}\) were observed during the
experiments where sulfate was added, which demonstrated that sulfate reduction
occurred in the microcosms\(^{48}\). In general, TCE is dechlorinated to cis-DCE under
iron-reduction or stronger reductive conditions. The cis-DCE is sequentially dechlorinated to VC under sulfate-reducing or methanogenic conditions. Finally, the VC is dechlorinated to ethylene under strong reductive methanogenic conditions\textsuperscript{29,49}. The ORP was around $-200$ to $-350$ mV for the batch experiments. This is advantageous for sulfate reduction and the subsequent methanogenesis, which leads to a more complete dechlorination of TCE\textsuperscript{49}. The results of the batch experiments show that the presence of sulfate improved VC dechlorination and had no adverse effect on TCE removal. Therefore, the sulfate that is generated by the persulfate oxidation procedure in the treatment train technology benefits the subsequent anaerobic bioremediation process.

At 100 d reaction time, the total number of soil bacteria slightly decreases from $1.3 \times 10^5$ to $4.62 \times 10^4$, $4.69 \times 10^4$, $4.42 \times 10^4$ and $4.32 \times 10^4$ CFU g\textsuperscript{-1} soil with the addition of 5,000, 10,000, 20,000 and 50,000 mg L\textsuperscript{-1} sulfate, respectively. Notably, there is no significant difference in the number of soil bacteria for each batch.

3.3 \textit{The effect of a bioremediation agent on anaerobic TCE degradation and the number of soil bacteria}
The effect of a commercial anaerobic bioremediation reagent, EcoClean, on anaerobic dechlorination of TCE was studied. Figure 6a shows the TCE degradation efficiency was 50, 97, 100, 100, and 100% with pseudo-first order rate constants of $7.4 \times 10^{-3}$, $3.95 \times 10^{-2}$, $4.38 \times 10^{-2}$, $4.52 \times 10^{-2}$ and $4.20 \times 10^{-2}$ d$^{-1}$ for the respective addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L$^{-1}$ EcoClean, after incubation time of 100 d. The results show that the addition of EcoClean enhanced the anaerobic dechlorination of TCE.

Figures 6b to 6e show that although higher concentrations of TCE degradation by-products were detected in some batches where the reagent was added due to more TCE dechlorination, the addition of EcoClean reduced the accumulation of cis-DCE and VC significantly. Generally, the concentration of TCE degradation by-products was lower in the batch where 5,000 mg L$^{-1}$ EcoClean was added than those where 10,000, 20,000 and 50,000 mg L$^{-1}$ EcoClean was added. This is because, in the TCE degradation process, the addition of a higher carbon source can lead to higher hydrogen generation, which increases the amount of other soil bacteria that compete with the dechlorination bacteria$^{26}$. Notably, at the end of the batch experiments, all of the TCE degradation by-products were completely removed in the sets where the
reagent was added. This demonstrates that EcoClean can enhance the complete
dechlorination of TCE. Since the addition of 5,000 to 10,000 mg L\(^{-1}\) of EcoClean
significantly enhanced the TCE biodegradation, EcoClean concentrations between
these dosages are suitable for the treatment train, to reduce the use of the reagent.

At 100 d reaction time, the total number of soil bacteria increases from \(1.3 \times 10^5\)
to \(1.39 \times 10^6, 1.69 \times 10^6, 4.02 \times 10^6, 6.22 \times 10^6\) and \(6.03 \times 10^6\) CFU g\(^{-1}\) soil for the
addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L\(^{-1}\) EcoClean, respectively. This
shows that in-situ microorganisms utilized the carbon source from EcoClean to
accelerate the anaerobic dechlorination.

3.4 The effect of a bioremediation reagent containing sulfate on anaerobic TCE
degradation and the number of soil bacteria

Figure 7a shows that with 5,000 mg L\(^{-1}\) EcoClean support, the TCE degradation
efficiency was 97, 94, 94, 94, and 96\% and the pseudo-first order rate constants were
\(3.95 \times 10^{-2}, 3.91 \times 10^{-2}, 3.68 \times 10^{-2}, 3.75 \times 10^{-2},\) and \(4.15 \times 10^{-2}\) d\(^{-1}\) for the addition of 0,
5,000, 10,000, 20,000 and 50,000 mg L\(^{-1}\) sulfate, respectively, after a reaction time of
100 d. There was no significant difference in the TCE degradation efficiency for these
batch experiments that added 0 to 50,000 mg L\(^{-1}\) sulfate. Therefore, sulfate concentrations ranging from 5,000 to 50,000 mg L\(^{-1}\), have no effect on TCE degradation for the concentration of EcoClean of 5,000 mg L\(^{-1}\). As shown in Figure 5b, sulfide concentrations, ranging from 0.7 to 1.0 mg L\(^{-1}\) were observed during the experiments, which demonstrated that more sulfate reduction occurred in the sulfate/EcoClean microcosms than in the microcosms where sulfate alone was added (Figure 5a).

Figures 7b to 7e show the production of TCE degradation byproducts during the experiments. As shown in Figure 7b, when sulfate was present in the system, the concentrations of cis-DCE decreased significantly. Since the concentrations of other TCE-degrading by-products were quite low and TCE degradation was dominated by the concentration of EcoClean, the effect of sulfate addition on the removal of those by-products was insignificant. It should be noted that sulfate played an important role in the removal of cis-DCE and VC in the batches with no EcoClean addition (Figures 4b and 4e). This indicates that the presence of sulfate can ensure the complete dechlorination of TCE when EcoClean is completely consumed. Accordingly, it is feasible to use persulfate followed by a bioremediation reagent as a treatment train
technology for TCE degradation. Notably, persulfate-releasing materials in the third
stage of the treatment train can be applied to further remove the by-products if the
accumulated concentrations of the residual by-products are high. In addition, at 100 d
reaction time, for 5,000 mg L\(^{-1}\) EcoClean support, the total number of soil bacteria
increases from \(1.3 \times 10^5\) to \(1.39 \times 10^6\), \(6.49 \times 10^6\), \(5.37 \times 10^6\), \(3.45 \times 10^6\) and \(1.43 \times
10^6\) CFU g\(^{-1}\) soil for the addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L\(^{-1}\) sulfate,
respectively. Therefore, the presence of sulfate did not significantly affect the
bacterial growth during the experiments.

3.5 The effect of a bioremediation reagent on DOC

DOC in the microcosms increased from 80 mg L\(^{-1}\) to approximately 4,500, 8,000,
17,500 and 46,000 mg L\(^{-1}\) for the addition of 5,000, 10,000, 20,000 and 50,000 mg L\(^{-1}\)
EcoClean reagent, respectively. During the TCE biodegradation process, the DOC
decreased as the reaction time increased, which shows that carbon was consumed by
the indigenous bacteria during the anaerobic dechlorination. The pseudo-first order
rate constants for DOC utilization during 100 d of incubation were \(2.2 \times 10^2\), \(1.9 \times
10^2\), \(1.0 \times 10^2\) and \(3.0 \times 10^3\) d\(^{-1}\) for the addition of 5,000, 10,000, 20,000, and 50,000
In order to determine the effect of sulfate on DOC degradation, DOC in the batches where EcoClean at 5,000 mg L\(^{-1}\) was added to different sulfate concentrations was also analyzed. The pseudo-first order rate constants for DOC utilization were \(2.2 \times 10^{-2}\), \(2.0 \times 10^{-2}\), \(1.6 \times 10^{-2}\), \(1.7 \times 10^{-2}\), and \(1.3 \times 10^{-2}\) d\(^{-1}\) for the addition of 0, 5,000, 10,000, 20,000, and 50,000 mg L\(^{-1}\) of sulfate, respectively. The rate constant slightly decreased as the sulfate concentration increased, which demonstrated that the presence of sulfate did not result in the consumption of more DOC. Aulenta et al.\(^5\) reported that the presence of sulfate caused a slightly higher degradation rate for the added substrate, butyrate, and decreased the rate of reductive dechlorination because there was rapid and competitive utilization of the electron donors by sulfate-reducing populations. However, no significant adverse impact on DOC consumption and TCE dechlorination was seen in this study. Therefore, more studies of different sites are required, in order to obtain more detailed information regarding the effect of sulfate on DOC consumption.
3.6 RT-PCR and DGGE analysis

Figure 8a shows the DGGE profiles for the PCR-amplified 16S rDNA during the experiments. The green bars refer to the bacteria that appear in background soil. The yellow bars refer to the bacteria that do not appear in the background soil. The red bars refer to the bacteria that match the bacteria in background soil. Lanes 1 and 6-9 in Figure 6a show that some bands in the background soil (Lane 1) disappeared, while some bacteria became significant, during 40 d of incubation. This demonstrates that the addition of sulfate and EcoClean causes significant changes in the microbial community. The bacterial community was also more abundant at Day 40 than that at Day 55 (Lanes 2-5). Since TCE concentrations were low at Day 55, the microbial activity may decrease, resulting in a reduction in bacterial abundance. In order to better understand the relationships between microorganisms in different microcosms, the UPGMA dendrogram of DGGE profiles was analyzed. Figure 8b shows that there was little similarity between the microbial community in most samples, which demonstrated that the addition of sulfate and EcoClean had a significant impact on the indigenous bacterial community because of the utilization of different electron acceptors by different predominant bacteria.
Previous studies have reported that *Dehalococcoides* degrade TCE to VC and ethane via reductive dechlorination \(^{25, 51, 52}\). The reductive dechlogenase, *vcrA*, of *Dehalococcoides* sp. is responsible for the complete dechlorination of TCE to ethane \(^{53}\). In this study, the RT-PCR technique was used to quantify the amounts of *Dehalococcoides* and *vcrA* genes. The RT-PCR results show that *Dehalococcoides* and *vcrA* genes were not detected at Day 0, while the number of *Dehalococcoides* was 3,423 gene copies/g in the presence of 5,000 mg L\(^{-1}\) EcoClean reagent, at 40 d reaction time. The existence of *Dehalococcoides* demonstrates the dechlorination capability of the treatment train technology that is used in this study. The number of *vcrA* genes was 1,105 gene copies/g in the presence of 5,000 mg L\(^{-1}\) EcoClean reagent and sodium sulfate, which is higher than the figure of 498 gene copies/g in the presence of 5,000 mg L\(^{-1}\) sodium sulfate, at 40 d reaction time. The addition of EcoClean reagent contributed to the increase in *Dehalococcoides* and *vcrA* genes, which are favorable for TCE degradation. The results of the RT-PCR analyses demonstrate that TCE can be completely dechlorinated at the contaminated site, because of the presence of dechlorinating bacteria and enzymes.
3.7 A Column experiment for the three-stage treatment train

Using the results of previous batch experiments, a continuous column experiment combined with chemical oxidation, anaerobic bioremediation and PRB treatment was used to determine the effectiveness of the treatment train technology on TCE degradation, for the first time. The concentrations of persulfate and EcoClean used were both 10,000 mg L\(^{-1}\), according to the results of the batch experiments. Figure 9 shows the variation in the TCE concentration in Columns 1 to 5. The average TCE concentration in the effluent of Column 1 was approximately 45 mg L\(^{-1}\), before persulfate was added. When 10,000 mg L\(^{-1}\) persulfate is added to Column 1, the TCE concentration decreased rapidly to 5 mg L\(^{-1}\) in 5 PV. The TCE concentration in the effluent of Column 1 decreased from 5.0 to 4.1 and 3.0 mg L\(^{-1}\) in the effluents of Columns 2 and 3, respectively. TCE was removed by in situ bacteria via reductive dechlorination degradation, which was demonstrated by the detection of 0.2 mg L\(^{-1}\) cis-DCE in the effluent of both Columns 2 and 3. After treatment by oxidant-releasing materials that continuously released approximately 6,000 mg L\(^{-1}\) of persulfate in Column 4, no TCE and its by-products were found in this section because of persulfate oxidation. Our previous studies have demonstrated that oxidant-releasing
materials were able to steadily release persulfate for at least 50 days\textsuperscript{22,23}. The PRB system in the last stage of the treatment train plays an important role in preventing the rebound of TCE and the accumulation of the TCE biodegrading byproducts, which ensures the success of site remediation. Tsai et al.\textsuperscript{2} applied a three-stage treatment train that was composed of surfactant flushing, permanganate oxidation, and aerobic biodegradation to treat TCE-contaminated groundwater (40 mg L\textsuperscript{-1}). Although TCE was removed effectively, their study was conducted by three separate static batch experiments. The continuous column test used in this study can simulate the application of the proposed treatment train in the field more appropriately. The results for the column experiment demonstrate that 88\% of TCE was removed in Column 1. This then accumulated to 94\% after the bioremediation treatment in Columns 2 and 3. 100\% of the TCE was removed after PRB treatment in Column 4. No TCE or its by-products were detected in the effluent of Column 5, which demonstrates that this treatment train technology is very effective.

In this study, chemical oxidation and anaerobic biodegradation technologies were employed simultaneously to remove TCE contamination in soil. Therefore, it is important to determine the difference in the ORP between these two technologies. For
example, a high ORP that is elevated by chemical oxidation can inhibit anaerobic
dechlorination, which generally requires a low ORP. In addition, a low ORP that is
caused by anaerobic bioremediation in Columns 2 and 3 can further affect the
oxidant-releasing material treatment in Column 4. An ORP of 10,000 mg L$^{-1}$
persulfate remained above 600 mV before it was injected to Column 1. The ORP of
the effluent in Column 1 decreased to 250 mV because there was a decrease in
persulfate concentration (30 mg L$^{-1}$). In Columns 2 and 3, the ORP decreased to lower
than -300 mV because of the decomposition of EcoClean by soil bacteria. This leads
to advantageous conditions for TCE dechlorination. ORP and persulfate
concentrations were around 500 mV and 4,000 mg L$^{-1}$, respectively, in the effluent of
Column 4, which contained oxidant-releasing materials. In Column 5, since persulfate
concentration decreased to 50 mg L$^{-1}$, a lower ORP (100 - 200 mV) was observed.
The column experiments show that there is no problem combining chemical oxidation
and anaerobic bioremediation in the treatment train technology for this study. The
results also show that ORP and persulfate concentration are important monitoring
parameters that determine the compatibility of each stage of the treatment train,
simply and rapidly, in the field.
The initial DOC was around 9,000 mg L\(^{-1}\) when EcoClean reagent was added to Columns 2 and 3. After 50 PV of reaction time, the DOC decreased to 4,000 mg L\(^{-1}\), which demonstrated that the reagent was consumed by soil bacteria during the anaerobic dechlorination of TCE. Column 1 had high persulfate concentration so the total number of bacteria decreased from \(1.23 \times 10^5\) to \(6.7 \times 10^4\) CFU g\(^{-1}\) soil. In Columns 2 and 3, when EcoClean reagent was added, the total number of bacteria recovered and increased to \(2.5 \times 10^5\) and \(2.6 \times 10^5\) CFU g\(^{-1}\) soil, respectively. Therefore, EcoClean reagent aids the growth of bacteria. In Column 5, the total number of soil bacteria slightly decreased to \(1.13 \times 10^5\) CFU g\(^{-1}\) soil due to the residual persulfate from Column 4. The results show that the proposed treatment train did not cause significant adverse effect on in situ bacteria.

It should be noted that the long-term effect of the treatment train on the bacterial community is an important issue. In a column study, Richardson et al.\(^{43}\) indicated that the diversity of soil bacterial community was reduced immediately after persulfate was injected. Although the microbial diversity increased after 30 days, it took 100 to 500 days for the recovery of phenanthrene-degrading bacterial groups. It is also necessary to confirm that the substrate addition can help TCE-degrading bacteria
maintain a good performance of TCE dechlorination during a long period of remediation\textsuperscript{54}. Therefore, the long-term effect of the treatment train operation on the microbial diversity and specific TCE-degrading bacteria such as \textit{Dehalococcoides} need to be further evaluated in a future study.

4. Conclusions

Soil and ground contamination is complex and difficult to remediate using a single technology. This study proposes a treatment train that comprises chemical oxidation, anaerobic bioremediation and PRB treatment to remediate TCE-contaminated groundwater. The results of the batch experiments show that a high concentration of TCE was oxidized effectively by persulfate oxidation. A low pH value and oxidative stress that is caused by the addition of persulfate may be the factor that inhibits the number of bacteria in soil. The results of the microcosm study demonstrate that the sulfate that is produced from persulfate oxidation could be utilized by indigenous bacteria to ensure the complete dechlorination of TCE, especially when no bioremediation reagent was present. The addition of EcoClean significantly enhanced the dechlorination of TCE. Dechlorinating bacteria,
Dehalococcoides, mainly contributed to the reductive dechlorination of TCE. The results of this study show that 10,000 mg L\(^{-1}\) of persulfate and the bioremediation reagent, and the PRB that can continuously release 6,000 mg L\(^{-1}\) of persulfate are suggested to operate the treatment train. The designed three-stage treatment train used low concentrations of persulfate coupled with the anaerobic bioremediation reagent to remove TCE and its degrading by-products completely without significant adverse effect on intrinsic microorganisms, which demonstrates that the proposed treatment train is a feasible technology for the remediation of groundwater that is contaminated with TCE. Future work should focus on the evaluation of the performance of TCE removal by lower persulfate concentrations and the long-term effect of the treatment train operation on the microbial community. The evaluation of the balance between cost and operation time should also be further investigated prior to field application.

Acknowledgement

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References


Table 1. The components of the batch experiments.

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<thead>
<tr>
<th>Batch experiment</th>
<th>Treatment</th>
<th>Constituent</th>
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<tr>
<td>Persulfate oxidation</td>
<td>Persulfate oxidation alone</td>
<td>TCE 50 mg L(^{-1}) + PS (0, 5,000, 10,000, 20,000 and 50,000 mg L(^{-1}) + aquifer sediment 10 g + DI water 50 mL</td>
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<td>Activated persulfate oxidation</td>
<td>TCE 50 mg L(^{-1}) + PS 5,000 mg L(^{-1}) (PS/Fe(II) molar ratios: 100/1, 100/10, and 100/20) + aquifer sediment 10 g + DI water 50 mL</td>
</tr>
<tr>
<td>Sterilized control</td>
<td>Anaerobic biodegradation</td>
<td>TCE 50 mg L(^{-1}) + HgCl(_2) 250 mg L(^{-1}) + aquifer sediment 10 g + DI water 50 mL</td>
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<tr>
<td>Anaerobic biodegradation</td>
<td>Anaerobic biodegradation with sulfate</td>
<td>TCE 5 mg L(^{-1}) + sulfate (0, 5,000, 10,000, 20,000 and 50,000 mg L(^{-1}) + aquifer sediment 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td>Anaerobic biodegradation with EcoClean</td>
<td>TCE 5 mg L(^{-1}) + EcoClean 5,000 mg L(^{-1}) + aquifer sediment 10 g + mineral medium 50 mL</td>
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<td>Anaerobic biodegradation with EcoClean + sulfate</td>
<td>TCE 5 mg L(^{-1}) + EcoClean 5,000 mg L(^{-1}) + sulfate (0, 5,000, 10,000, 20,000 and 50,000 mg L(^{-1}) + aquifer sediment 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td>Sterilized control</td>
<td>TCE 5 mg L(^{-1}) + HgCl(_2) 250 mg L(^{-1}) + aquifer sediment 10 g + mineral medium 50 mL</td>
<td></td>
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Figure captions

Figure 1. The layout of the column experiments.

Figure 2. The effect of persulfate concentration on TCE degradation: (a) TCE removal using different persulfate concentrations; (b) the residue of persulfate for different persulfate concentrations; and (c) pH variation.

Figure 3. The effect of ferrous ion concentration on TCE degradation: (c) TCE removal for different ferrous ion concentrations and (d) the residue of persulfate for different ferrous ion concentrations; and (c) pH variation.

Figure 4. The effect of sulfate concentration on (a) TCE degradation; (b) cis-DCE production; (c) trans-DCE production; (d) 1,1-DEC production and (e) VC production.

Figure 5. The production of sulfide in (a) sulfate addition and (b) EcoClean/sulfate addition systems.
Figure 6. The effect of EcoClean concentration on (a) TCE degradation; (b) cis-DCE production; (c) trans-DCE production; (d) 1,1-DEC production and (e) VC production.

Figure 7. The effect of sulfate concentration on (a) TCE degradation; (b) cis-DCE production; (c) trans-DCE production; (d) 1,1-DEC production and (e) VC production, in the presence of EcoClean.

Figure 8. The results of DGGE analysis for the batch anaerobic biodegradation experiments: (a) the DGGE profiles for the PCR-amplified 16S rDNA and (b) the similarity in the DGGE profiles.

Figure 9. The variation in TCE concentration in the column test for (a) Column 1 and (b) Columns 2-4.
Figure 1
Figure 2

(a) TCE (C/C₀) vs. Time (h) for different PS concentrations.

(b) PS (C/C₀) vs. Time (h) for different PS concentrations.

(c) pH vs. Time (h) for different PS concentrations.
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7

(a) 

(b) 

(c) 

(d) 

(e)
Figure 8

(a) 1: Background soil
2: EcoClean 0.5% @ 55 d
3: EcoClean 2% @ 55 d
4: EcoClean 0.5% + sulfate 0.5% @ 55 d
5: Sulfate 0.5% @ 55 d
6: EcoClean 0.5% @ 40 d
7: EcoClean 2% @ 40 d
8: EcoClean 0.5% + sulfate 0.5% @ 40 d
9: Sulfate 0.5% @ 40 d

(b) Sulfate 0.5% @ 40 d
Sulfate 0.5% @ 55 d
Background soil
EcoClean 2% @ 40 d
EcoClean 0.5% @ 55 d
EcoClean 0.5% @ 40 d
EcoClean 0.5% + sulfate 0.5% @ 40 d
EcoClean 0.5% + sulfate 0.5% @ 55 d
EcoClean 2% @ 55 d
Figure 9

(a) Column 1

(b) Column 2, Column 3, Column 4, Column 5
Graphic abstract

The proposed treatment train removed TCE and its byproducts effectively and there was no problem with the connection of chemical oxidation and anaerobic bioremediation in the novel treatment train technology.