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

3

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17 **Abstract**

18 This study used a novel three-stage treatment train that was composed of chemical
19 oxidation, anaerobic bioremediation and passive reactive barrier to remediate
20 trichloroethylene (TCE)-contaminated groundwater. Batch oxidation and
21 biodegradation experiments and a continuous column study were used to evaluate the
22 compatibility of different technologies and the feasibility of the removal of TCE by
23 the treatment train. The results of batch experiments show that high concentrations of
24 TCE (50 mg L^{-1}) were removed completely by the addition of 5,000 to 50,000 mg L^{-1}
25 persulfate during 24 to 96 h of reaction. Ferrous ion-activated persulfate may result in
26 a residue of TCE due to the rapid consumption of persulfate by ferrous ions.
27 Significant inhibition of soil bacteria was observed upon the addition of persulfate in
28 concentrations greater than 20,000 mg L^{-1}. Both low pH and the oxidative stress of
29 persulfate were responsible for the adverse effect on indigenous microorganisms.
30 The results of a microcosm study reveal that the presence of high concentrations of
31 sulfate (up to 50,000 mg L^{-1}) had no adverse effect on TCE removal. Sulfate
32 significantly enhanced the dechlorination of vinyl chloride via sulfate reduction,
33 which demonstrates that sulfate produced from persulfate oxidation could be utilized

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34 by indigenous bacteria to achieve the complete dechlorination of TCE. The addition
35 of 5,000 to 50,000 mg L^{-1} bioremediation reagent improved the degradation of TCE.
36 Dechlorinating bacteria, *Dehalococcoides*, and the reductive dechlogenase, *vcr*A, of
37 *Dehalococcoides* were detected during TCE biodegradation. The results of a column
38 study show that the proposed treatment train removed TCE and its byproducts
39 effectively and there was no problem with the connection of chemical oxidation and
40 anaerobic bioremediation in the novel treatment train technology. The use of 10,000
41 mg L^{-1} of persulfate and the bioremediation reagent, and the PRB that can
42 continuously release 6,000 mg L^{-1} of persulfate are suggested to operate the treatment
43 train. The proposed treatment scheme will provide a more effective alternative for the
44 remediation of contaminated sites in the future.

45

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

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48 ## 1. Introduction

49 Soil and groundwater contamination is ubiquitous and usually difficult to treat
50 because of complex geological, biological, hydraulic and pollution conditions in sites.
51 Treatment trains are used when no single technology is capable of treating all of the
52 contaminants in a particular medium [1]. Two or more innovative and established
53 technologies can be used together in treatment trains, which are either integrated
54 processes or a series of treatments that are combined in sequence to provide the
55 necessary treatment [2]. Many technologies, such as bioremediation, electrochemical
56 treatment, photocatalytic oxidation, chemical oxidation/reduction, permeable reactive
57 barriers and ultrasonication, have been used for treatment trains to remediate
58 contamination in soil and groundwater[2-7]. The synergy can achieve results that are
59 better than the sum of the effect of the individual technologies.

60 Trichloroethylene (TCE) is widely used as a cleaning agent for industrial metals,
61 metal degreasing and dry cleaning operations and is one of the common contaminants
62 that is observed in soil and groundwater[2, 8]. Of the various treatment methods, in situ
63 chemical oxidation (ISCO) is a widely used remediation technology for the in-situ
64 treatment of contaminated soils and groundwater. Common ISCO oxidants that are

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65 used for the remediation of contaminated sites include hydrogen peroxide (H_2O_2),

66 such as Fenton's reagent and Fenton-like reaction, ozone (O_3), and permanganate

67 (MnO_4^-) [9]. Persulfate (PS) ($S_2O_8^{2-}$) is a newer oxidant that can be activated to

68 promote the formation of sulfate free radicals ($SO_4^-\cdot$), which are instrumental in the

69 destruction of chlorinated solvents such as chlorinated ethanes and chlorinated

70 ethenes [8, 10-15]. Persulfate is a strong oxidant with a high redox potential of 2.01 V for

71 the half-cell reaction that is shown below [16]:

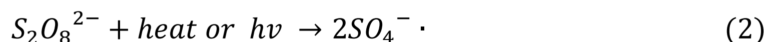
$$S_2O_8^{2-} + 2e^- \rightarrow 2SO_4^{2-}, \qquad E^0 = 2.01\,V \qquad (1)$$

72 Persulfate can be activated by various activators to form more powerful sulfate free

73 radicals ($SO_4^-\cdot$), which have a higher redox potential of 2.60 V [17-19]. When heat or UV

74 light is applied, one mole of persulfate produces two moles of sulfate free radicals, as

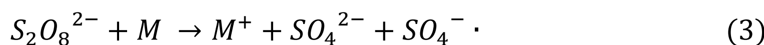
75 shown in reaction (2):



$$S_2O_8^{2-} + heat\ or\ hv \rightarrow 2SO_4^-\cdot \qquad (2)$$

76 With the activation of transition metals (represented by M), one mole of persulfate

77 produces one mole of sulfate free radicals:



$$S_2O_8^{2-} + M \rightarrow M^+ + SO_4^{2-} + SO_4^-\cdot \qquad (3)$$

78 The half-cell reaction for sulfate free radicals is:

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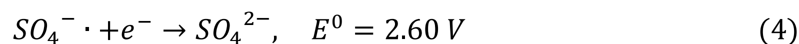

$$SO_4^{-}\cdot + e^{-} \rightarrow SO_4^{2-}, \quad E^0 = 2.60\,V \qquad (4)$$

79 Persulfate oxidation is used to degrade various contaminants, such as chlorinated
80 organic compounds and petroleum hydrocarbons[20, 21]. Recently, persulfate-releasing
81 materials that are composed of persulfate, cement and sand have been successfully
82 developed to remediate groundwater that is contaminated. The persulfate-releasing
83 materials can form an ISCO barrier system that releases persulfate for a long period of
84 time, which results in continuous degradation of contaminants in the subsurface [22, 23].
85 Although ISCO treatment is effective for the remediation of contaminated sites,
86 the cost of ISCO may be high due to the expense of the oxidant. In addition, when the
87 concentrations of the contaminants are low, most of the oxidants added to the
88 subsurface may be substantially consumed by natural organic matter. Consequently,
89 to ensure better remediation, treatment trains such as chemical oxidation coupled with
90 bioremediation have been proposed [24]. Bioremediation is an environmentally friendly
91 and cost-effective remedial technology. However, TCE is not biodegraded by direct
92 metabolism under aerobic conditions. Although TCE can be removed via aerobic
93 co-metabolism, TCE biodegradation appears to be more effective under anaerobic
94 conditions [25, 26]. In situ bacteria, including nitrate-, iron- and sulfate-reducing bacteria

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95 and methanogens remove TCE via reductive dechlorination under anaerobic
96 conditions [27-29]. It has been reported that the dechlorination of TCE is enhanced by
97 sulfate-reducing bacteria under anaerobic conditions [29, 30]. Koenigsberg[31] indicated
98 that sulfate-reducing conditions are the predominant microbiological conditions
99 during the enhanced biological treatment of chlorinated hydrocarbon-contaminated
100 groundwater using Hydrogen Release Compound (HRC™). It is noteworthy that
101 although the degradation of TCE can be achieved under anaerobic conditions,
102 dichloroethene (DCE) and more toxic vinyl chloride (VC) may be produced and can
103 accumulate during the reductive dechlorination of TCE [32].
104 As shown in Reaction (1), two moles of sulfate are produced when one mole of
105 persulfate is consumed. Since sulfate is an electron acceptor for anaerobic
106 biodegradation, it may be feasible to use persulfate followed by anaerobic
107 bioremediation to enhance the removal of TCE in the subsurface. However, before the
108 combined remedial scheme is applied, it is necessary to evaluate the effects of sulfate
109 on the biodegradation of TCE and the compatibility of chemical oxidation and
110 anaerobic bioremediation. The accumulation of DCE and VC during anaerobic
111 dechlorination of TCE must also be considered.

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112 While high oxidant concentrations (10-30% wt/wt) are usually used in field
113 application[33, 34], this study attempts to apply lower oxidant concentrations coupled
114 with an anaerobic biodegradation to treat contaminated groundwater. A three-stage
115 treatment train that was composed of persulfate oxidation, anaerobic bioremediation
116 and passive reactive barrier (PRB) was developed to remediate TCE-contaminated
117 groundwater. The main objectives of this study were to: (1) evaluate the efficiency of
118 persulfate oxidation and anaerobic bioremediation in TCE removal; (2) assess the
119 effect of sulfate concentrations on the anaerobic biodegradation of TCE; (3) evaluate
120 the feasibility of combining persulfate oxidation, anaerobic bioremediation and
121 persulfate-releasing materials for the remediation of a TCE-contaminated aquifer; (4)
122 determine the optimal operational conditions for the three-stage treatment train system
123 and (5) evaluate the effects of the proposed treatment train on indigenous microbes.
124

125 **2. Materials and Methods**

126 *2.1 Chemicals and materials*

127 The chemicals that were used for this study are: TCE (99.9%, J. T. Baker, USA),
128 cis-DCE (>99%, Tokyo Chemistry Industrial Co., Japan), trans-DCE (>98%, Tokyo

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129 Chemistry Industrial Co., Japan), 1,1-DCE (> 99%, Merck, USA), VC (2,000 ppm in
130 methanol, Supelco, USA) and sodium persulfate (> 99%, Riedel-de Haen, Germany).
131 An anaerobic bioremediation reagent, EcoClean^{TM}, which is composed of
132 hydrocarbons and amino acids, was purchased from Ecocycle Co., Japan.
133 Persulfate-releasing materials that release oxidant continuously were synthesized
134 similarly to the method of a previous study by the authors [23]. The mass ratio of
135 persulfate/cement/sand/water for the persulfate-releasing materials was 1/1.4/0.24/0.7.
136 Aquifer sediments and groundwater samples used for the experiments were collected
137 from a TCE-contaminated site in southern Taiwan. All of the samples were stored at
138 $4^{o}C$ before use. The values for pH, moisture content, total organic carbon (TOC),
139 cation exchange capacity (CEC), oxidation-reduction potential (ORP) and the total
140 bacteria of the soil were 7.54, 16%, 2.56%, 8.19 meq/100 g, 98 mV and 1.3×10^5
141 CFU g^{-1} soil, respectively. A mineral medium that contained buffer solution, calcium
142 and magnesium solutions, and trace elements was used for anaerobic biodegradation
143 experiments [35].
144
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146 *2.2 Batch oxidation experiments*

147 Batch oxidation experiments were conducted using 60 mL serum bottles for
148 different persulfate concentrations and PS/Fe(II) ratios, in order to evaluate the
149 appropriate conditions for TCE oxidation. Each bottle was filled with 10 g of the
150 aquifer soil and 50 mL DI water with the required TCE, persulfate, and ferrous ion
151 concentrations. A set without persulfate addition was constructed to evaluate the
152 possible biodegradation of TCE during the oxidation experiments. Control bottles
153 containing 250 mg L^{-1} of $HgCl_2$ were autoclaved twice before use. All batch
154 experiments were conducted in duplicate and kept at 25 ± 2°C in darkness, until
155 analysis. The detailed components of the batch oxidation experiments are shown in
156 Table 1.

157

158 *2.3 Batch anaerobic biodegradation experiments*

159 A microcosm study was conducted using 60 mL serum bottles, in order to assess
160 the ability of intrinsic bacteria to dechlorinate TCE and to determine the effect of
161 EcoClean and sulfate concentration on TCE biodegradation under anaerobic
162 conditions. The effect of sulfate concentration on TCE removal in the presence of

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163 EcoClean was also determined. Microcosms contained 10 g of the aquifer soil as the
164 sources of microorganisms and 50 mL mineral nutrients with the required TCE,
165 EcoClean, and sulfate concentrations. Aquifer soil and the mineral nutrients were
166 purged with N_2 to eliminate O_2 before use. The headspace of each serum bottle was
167 filled with N_2 to keep the microcosm anoxic. Control bottles containing 250 mg L^{-1} of
168 $HgCl_2$, and inocula for the control groups, were autoclaved twice before use. pH in all
169 microcosms was around 6.5-7 during the experiments. All of the microcosm studies
170 were conducted in duplicate and kept at 25 ± 2°C in darkness, until analysis. The
171 detailed components of the microcosms are shown in Table 1.
172

173 *2.4 Column study*

174 A column study was performed to evaluate the ability of the proposed three-stage
175 treatment train to remediate TCE-contaminated groundwater. The treatment train that
176 was used in this study consisted of persulfate oxidation, anaerobic bioremediation
177 reagent, and a PRB (persulfate-releasing materials). A total of five continuous-flow
178 glass columns were used to simulate the treatment train system. Figure 1 shows the
179 layout of the column experiments. Five columns were used in sequence; i.e.,

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180 persulfate oxidation for the first column, anaerobic bioremediation for the second and
181 the third columns, PRB treatment for the fourth column and the fifth column was used
182 for monitoring. Each glass column was 30-cm long, with an inner diameter of 5 cm.
183 All columns were filled with in situ aquifer sediment that was sampled from a
184 TCE-contaminated site in southern Taiwan, except for Column 4. In situ groundwater
185 with 50 mg L^{-1} of TCE (spiked) and 10,000 mg L^{-1} of persulfate solution were stored
186 in two gas-sampling bags, in order to prevent the TCE from becoming volatile and to
187 prevent water evaporation during the experiment. TCE and persulfate solutions were
188 continuously pumped into the columns by a peristaltic pump. The flow rate and
189 average pore volume for the column study were 0.24 mL/min and 178 mL,
190 respectively.
191 Column 1 simulated the first stage of the treatment train system. It was expected
192 that high concentrations of TCE would decrease rapidly and significantly in Column 1.
193 The result of a pre-test showed that the output TCE concentration for Column 1 was 5
194 mg L^{-1}, after the injection of persulfate. Therefore, Columns 2 and 3 were pre-filled
195 with 5 mg L^{-1} TCE contaminated soil, followed by a concentration of 10,000 mg L^{-1}
196 of EcoClean reagent, in order to evaluate the effectiveness of EcoClean on the

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197 anaerobic reductive dechlorination of low concentrations of TCE from Column 1. In
198 Column 4, a PRB that contained 480 g persulfate-releasing materials which can
199 continuously release persulfate with an average concentration of 6,000 mg L^{-1} was
200 constructed to oxidize the residual TCE, DCEs, and VC from Columns 2 and 3.
201 Column 5 represented the down-gradient area of the remedial system.
202
203 *2.5 Microbial analysis*
204 A total heterotrophic count was performed to determine the effect of persulfate
205 and EcoClean on intrinsic bacteria, during all experiments. A Difco™ plate count agar
206 (Becton, Dickinson and Co., USA) was used to assess the total number of bacteria in
207 sediment samples, using the spread plate method [36]. During the batch anaerobic
208 biodegradation experiments, polymerase chain reaction-denaturing gradient gel
209 electrophoresis (PCR-DGGE) was used to detect the variation in the bacterial
210 community. DNA was extracted from 0.5 g soil samples using a PowerSoil® DNA
211 Isolation Kit (Mo Biol, USA). The V6-V8 region of the 16S rDNA was amplified
212 using the primers, 968F (5'-AAC GCG AAG AAC CTT AC-3') and 1401R (5'-CGG
213 TGT GTA CAA GAC CC-3'). PCR amplification used an initial denaturation at 97 °C

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214 for 5 min and then 28 cycles of annealing at 95 °C for 1 min, 54 °C for 40 sec and 72
215 °C for 30 sec, followed by a final extension at 72 °C for 7 min. Each amplified PCR
216 product underwent DGGE using a Bio-Rad DCode system (Bio-Rad, Hercules, CA,
217 USA), in order to monitor the changes in the microbial diversity during the
218 experiments. A 10% polyacrylamide gel with a 40-60% denaturant gradient
219 underwent electrophoresis at 60 °C and 65 V for 870 min. After electrophoresis, the
220 gels were stained using the silver-stain method. Quantity One 4.6.8 software (BioRad,
221 USA) was used to analyze DGGE banding patterns. Dendrograms were created using
222 the algorithm for the unweighted pair-group method using arithmetic averages
223 (UPGMA) for cluster analysis. Real-time (RT)-PCR (quantitative PCR, qPCR) was
224 performed using a LightCycler® 480 (Roche, Germany) to detect the expression of
225 *Dehalococcoides* (968F: ACG TGC CAG CAG CCG CGG TA; 1401R: TCC TCC
226 CCG TTT CGC GGG GCA) and the reductive dechlogenase, *vcr*A, of
227 *Dehalococcoides* (968F: TGC TGG TGG CGT TGG TGC TCT; 1401R: TGC CCG
228 TCA AAA GTG GTA AAG). All of parameters for these procedures, including the
229 annealing and polymerization temperatures, the primer concentrations and the $MgCl_2$
230 concentration for qPCR, followed the recommendations given in the procedures of the

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231 manufactures.

232

233 *2.6 Analytical methods*

234 Aqueous samples were pretreated using a purge and trap equipment and then
235 analyzed for TCE, cis-DCE, trans-DCE, 1,1-DCE and VC using a gas
236 chromatography (7890A, Agilent Technologies, USA) equipped with a flame
237 ionization detector and capillary column (GsBP-624, 60 m × 0.32 mm). The operating
238 temperatures were maintained at 180°C for the injector and 230°C for the detector.
239 The oven temperature was initially maintained at 35°C for 5 min, then elevated at a
240 rate of of 11°C min^{-1} to 115°C, and held at 115°C for 3 min. The temperature was then
241 raised at a rate of 20°C min^{-1} to 220°C, and then maintained at 220°C for 1 min.
242 Sulfide and persulfate were respectively analyzed using a spectrophotometer (DR
243 5000, Hach Company, USA) according to Methylene-Blue method [36] and the method
244 of Huang et al. [16]. Dissolved organic carbon (DOC) was analyzed using a total carbon
245 analyzer (Phoenix 8000, Tekmar Dohrmann, USA) [36]. The pH and redox potential
246 values were measured using a pH meter (SUNTEX SP-2200, Taiwan) and an ORP
247 meter (ORION Model 250A+, Thermo Fisher Scientific), respectively.

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248

249 **3. Results and discussion**

250 *3.1 The effect of persulfate and ferrous ion concentrations on TCE degradation and*

251 *the number of soil bacteria*

252 Figure 2a shows that during 24 to 96 h of reaction, 100% of the TCE was removed

253 using different persulfate dosages. The results of the experiment without persulfate

254 addition show that TCE was not biodegraded during 96 h of incubation. Therefore, in

255 situ microorganisms did not contribute to the removal of TCE during the oxidation

256 experiments. The DO concentrations in the bottles were around 5.7 mg L^{-1} during the

257 experiment. Although TCE may be biodegraded via cometabolic biodegradation using

258 soil organic matter as the carbon source under aerobic conditions[37], aerobic TCE

259 biodegradation was not observed in this study. Longer incubation time may be needed

260 to evaluate the potential of intrinsic TCE biodegradation under aerobic conditions.

261 The pseudo-first order rate constants were 9.05×10^{-2}, 1.89×10^{-1}, 3.39×10^{-1} and

262 3.73×10^{-1} h^{-1} for the addition of 5,000, 10,000, 20,000 and 50,000 mg L^{-1} persulfate,

263 respectively. The TCE degradation rate increased as the amount of persulfate that was

264 added increased. Liang et al.[38] and Fang et al.[39] found that sulfate radicals were

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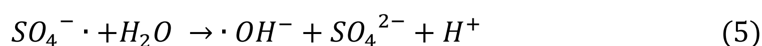
265 predominant under acid and neutral conditions at ambient temperature (10-30 °C). In

266 this study, the temperature and pH in the persulfate systems were 25 °C and 4 to 6.5,

267 respectively. This indicates that sulfate radicals mainly contributed the removal of

268 TCE. In addition, sulfate radicals can react with water to produce hydroxyl radicals

269 under all pH conditions, as shown in reaction (5)[40]:



$$SO_4{}^{-}\cdot + H_2O \rightarrow \cdot OH^{-} + SO_4{}^{2-} + H^{+} \qquad (5)$$

270 Since hydroxyl radicals preferred to attack unsaturated double bond in TCE[12],

271 The produced hydroxyl radicals may also play a role in the degradation of TCE. It

272 should be noted that although it is not difficult to treat TCE in the bench-scale

273 systems, the rebound of contaminants was most prevalent at chlorinated

274 solvent-contaminated sites applying chemical oxidation[41]. Therefore, more efforts

275 (e.g., treatment trains) need to be implemented to prevent rebound occurrence in the

276 field. Figure 2b shows that more than 70% of persulfate remained in the system at the

277 end of the experiments, which demonstrated that persulfate was quite persistent. Since

278 the applied persulfate concentrations removed TCE effectively during a short time of

279 reaction, a study using persulfate less than 5,000 mg L^{-1} could be performed in the

280 future to make a balance between cost and time. The results show that persulfate

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281 treatment rapidly decreased high concentrations of TCE so it is a suitable first stage in
282 the treatment train.

283 The total number of soil bacteria changed from an original value of 1.3×10^5 to
284 1.4×10^5, 2.6×10^4, 1.3×10^3, 1.3×10^2 and 6.8×10^1 CFU g^{-1} soil upon the respective
285 addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L^{-1} persulfate, at 96 h reaction
286 time. The inhibition of soil bacteria increased as the amount of persulfate that was
287 added increased. As shown in Figure 2c, after the addition of persulfate, especially for
288 the 20,000 and 50,000 mg L^{-1} persulfate batches, the pH decreased from 7 to 5.1 and
289 4.3, respectively, which was the main reason for the inhibition of the total soil
290 bacteria. In general, the persulfate reaction results in a pH decrease in soil and/or
291 aquifers that have a low buffering capacity. Decreases ranging from 2.5 to 6 pH units
292 have been observed under laboratory conditions, for both aqueous [8, 20] and soil slurry
293 experiments [42]. Tsitonaki et al.[42] and Richardson et al.[43] found that significant
294 decreases in microbial density were observed after exposure to persulfate due to the
295 drop of pH. Our findings are consistent with the above studies. It should be noted that
296 although the pH was about 6.5 in the experiments where 5,000 and 10,000 mg L^{-1}
297 persulfate was added, the number of bacteria also decreased. This phenomenon

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298 demonstrates that in addition to pH, the oxidative stress of persulfate also has an

299 adverse effect on indigenous microorganisms. Sutton et al. [33] also indicated that

300 oxidative stress from chemical oxidation had an effect on soil bacteria. The decrease

301 in the total number of soil bacteria may be due to the presence of sulfate radicals in

302 the system. In addition, persulfate alone may also kill bacteria directly. Dogan-Subasi

303 et al.[44] evaluated the effect persulfate on microbial dechlorination activity under

304 different persulfate concentrations (0.01-4.52 g/L). No gene expression and cell

305 activity were detected with the addition of 1.13-4.25 g/L persulfate. Since the

306 decrease in TCE concentration was not observed under these persulfate concentrations,

307 the author concluded that persulfate was not activated in the experiments. It has been

308 demonstrated that the oxidative stress caused by chlorine, permanganate, and

309 hydrogen peroxide can inactivate bacteria[45, 46]. The redox potential of persulfate is

310 2.01 V, which is higher than that of chlorine (1.4 V), permanganate (1.7 V), and

311 hydrogen peroxide (1.8 V)[34]. Therefore, the oxidative stress caused by both persulfate

312 and sulfate radicals contributed to the inhibition of bacterial growth in the

313 experiments. Since adding persulfate at concentrations of 5,000 and 10,000 mg L^{-1}

314 removed TCE without causing severe damage to in situ bacteria, persulfate

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315 concentrations between 5,000 and 10,000 mg L^{-1} were selected for the treatment train
316 in the following experiments.
317 Ferrous ions serve as electron donors that activate persulfate to generate sulfate
318 radicals (see Eq. (3)). Therefore, the degradation of TCE by ferrous ion
319 (Fe(II))-activated PS was studied, in order to better understand the effect of the
320 PS/Fe(II) molar ratio on TCE removal. Figure 3a shows the efficiency with which
321 TCE was removed by ferrous ion-activated persulfate. After 96 h of reaction time,
322 TCE is completely removed by persulfate alone and using PS/Fe(II) molar ratios of
323 100/1 and 100/10. When PS/Fe(II) with molar ratio of 100/20 was added, only 80% of
324 TCE was removed, which was a lower figure than that for the unactivated persulfate
325 batch. Figure 3b shows that the ferrous ions in the PS/Fe(II) with molar ratio of
326 100/20 were totally consumed within 24 h of reaction, which resulted in the
327 incomplete removal of TCE. The pseudo-first order rate constants were 9.05×10^{-2},
328 48.6×10^{-2}, 190.5×10^{-2} and 4.24×10^{-2} h^{-1} for the addition of persulfate alone and
329 PS/Fe(II) with molar ratios of 100/1, 100/10 and 100/20, respectively. The results
330 show that although TCE removal was enhanced by ferrous ion-activated persulfate,
331 there can be a residue of the contaminant because of the excess consumption of

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332 persulfate by ferrous ions. Liang et al.[8] used persulfate that was activated by ferrous
333 ions to degrade TCE. Their results show that organic contaminants are more
334 efficiently destroyed by sequentially adding controlled amounts of ferrous ions.
335 However, if ferrous ion levels become excessive, it appears that ferrous ions scavenge
336 sulfate free radicals, which results in a decrease in the efficiency with which
337 contaminants are destroyed [8]. Chen et al. [47] also reported that excess addition of
338 ferrous ion causes a decrease in the rate of degradation of methyl tert-butyl ether
339 (MTBE) because there is competition for sulfate free radicals between ferrous ions
340 and MTBE. Therefore, an appropriate dosage of ferrous ion needs to be selected when
341 ferrous-ion activated persulfate is applied. Figure 3b also shows that large amounts of
342 persulfate were consumed when ferrous ions were added. Although TCE is removed
343 more efficiently when an appropriate amount of ferrous ions is added to the persulfate
344 oxidation system, there is additional consumption of persulfate. Therefore, if the
345 remediation time is allowed, the addition of ferrous ion may not be necessary, which
346 would eliminate the need and cost of further persulfate injection.

347 As shown in Figure 3c, after PS/Fe(II) with molar ratios of 100/1, 100/10, and
348 100/20 were added, the pH decreased from 7 to 6.1, 6.1, and 5.9, respectively. The

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349 final pH in the PS/Fe(II) batches was slightly lower than that in the persulfate alone
350 batch (pH 6.5) due to the addition of acid ferrous ions. The total soil bacteria number
351 decreased from 1.3×10^5 to 2.65×10^4, 2.78×10^4, 3.59×10^4 and 2.56×10^4 CFU g^{-1} soil
352 for the addition of persulfate alone and PS/Fe(II) with molar ratios of 100/1, 100/10
353 and 100/20, respectively, at 96 h reaction time. These experimental results show that
354 both non-activated persulfate and ferrous iron-activated persulfate have an effect on
355 the total number of soil bacteria.
356

357 *3.2 The effect of sulfate concentration on the anaerobic biodegradation of TCE and*
358 *the number of soil bacteria*

359 Sulfate (SO_4^{2-}) is one of the persulfate oxidation products which may affect the
360 biodegradation of TCE and the survival of soil bacteria. Therefore, the effect of
361 sulfate on anaerobic TCE biodegradation was studied. Figure 4a shows that, after 100
362 d reaction time, 50, 55, 52, 53 and 50% of the TCE was biodegraded in the batches
363 containing 0, 5,000, 10,000, 20,000 and 50,000 mg L^{-1} of sulfate, respectively. The
364 pseudo-first order rate constants for TCE degradation by in-situ bacteria were $7.4 \times$
365 10^{-3}, 8.4×10^{-3}, 8.3×10^{-3}, 7.8×10^{-3} and 8.0×10^{-3} d^{-1}, respectively. The results

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366 indicate that indigenous microorganisms at the contaminated site were capable of
367 degrading TCE. All of the batch experiments show that a similar amount of TCE was
368 removed. Therefore, the sulfate generated by persulfate oxidation process in the
369 treatment train technology has no effect on anaerobic TCE degradation by indigenous
370 bacteria.

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

379 Figure 5a shows the production of sulfide in sulfate addition systems. Significant
380 sulfide concentrations ranging from 0.35 to 0.80 mg L^{-1} were observed during the
381 experiments where sulfate was added, which demonstrated that sulfate reduction
382 occurred in the microcosms[48]. In general, TCE is dechlorinated to cis-DCE under

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383 iron-reduction or stronger reductive conditions. The cis-DCE is sequentially
384 dechlorinated to VC under sulfate-reducing or methanogenic conditions. Finally, the
385 VC is dechlorinated to ethylene under strong reductive methanogenic conditions [29, 49].
386 The ORP was around −200 to −350 mV for the batch experiments. This is
387 advantageous for sulfate reduction and the subsequent methanogenesis, which leads to
388 a more complete dechlorination of TCE [49]. The results of the batch experiments show
389 that the presence of sulfate improved VC dechlorination and had no adverse effect on
390 TCE removal. Therefore, the sulfate that is generated by the persulfate oxidation
391 procedure in the treatment train technology benefits the subsequent anaerobic
392 bioremediation process.
393 At 100 d reaction time, the total number of soil bacteria slightly decreases from
394 1.3×10^5 to 4.62×10^4, 4.69×10^4, 4.42×10^4 and 4.32×10^4 CFU g^{-1} soil with the
395 addition of 5,000, 10,000, 20,000 and 50,000 mg L^{-1} sulfate, respectively. Notably,
396 there is no significant difference in the number of soil bacteria for each batch.
397
398 *3.3 The effect of a bioremediation agent on anaerobic TCE degradation and the*
399 *number of soil bacteria*

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400 The effect of a commercial anaerobic bioremediation reagent, EcoClean, on
401 anaerobic dechlorination of TCE was studied. Figure 6a shows the TCE degradation
402 efficiency was 50, 97, 100, 100, and 100% with pseudo-first order rate constants of
403 7.4×10^{-3}, 3.95×10^{-2}, 4.38×10^{-2}, 4.52×10^{-2} and 4.20×10^{-2} d^{-1} for the respective
404 addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L^{-1} EcoClean, after incubation
405 time of 100 d. The results show that the addition of EcoClean enhanced the anaerobic
406 dechlorination of TCE.

407 Figures 6b to 6e show that although higher concentrations of TCE degradation
408 by-products were detected in some batches where the reagent was added due to more
409 TCE dechlorination, the addition of EcoClean reduced the accumulation of cis-DCE
410 and VC significantly. Generally, the concentration of TCE degradation by-products
411 was lower in the batch where 5,000 mg L^{-1} EcoClean was added than those where
412 10,000, 20,000 and 50,000 mg L^{-1} EcoClean was added. This is because, in the TCE
413 degradation process, the addition of a higher carbon source can lead to higher
414 hydrogen generation, which increases the amount of other soil bacteria that compete
415 with the dechlorination bacteria [26]. Notably, at the end of the batch experiments, all of
416 the TCE degradation by-products were completely removed in the sets where the

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417 reagent was added. This demonstrates that EcoClean can enhance the complete
418 dechlorination of TCE. Since the addition of 5,000 to 10,000 mg L^{-1} of EcoClean
419 significantly enhanced the TCE biodegradation, EcoClean concentrations between
420 these dosages are suitable for the treatment train, to reduce the use of the reagent.
421 At 100 d reaction time, the total number of soil bacteria increases from 1.3×10^5
422 to 1.39×10^6, 1.69×10^6, 4.02×10^6, 6.22×10^6 and 6.03×10^6 CFU g^{-1} soil for the
423 addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L^{-1} EcoClean, respectively. This
424 shows that in-situ microorganisms utilized the carbon source from EcoClean to
425 accelerate the anaerobic dechlorination.
426
427 *3.4 The effect of a bioremediation reagent containing sulfate on anaerobic TCE*
428 *degradation and the number of soil bacteria*
429 Figure 7a shows that with 5,000 mg L^{-1} EcoClean support, the TCE degradation
430 efficiency was 97, 94, 94, 94, and 96% and the pseudo-first order rate constants were
431 3.95×10^{-2}, 3.91×10^{-2}, 3.68×10^{-2}, 3.75×10^{-2}, and 4.15×10^{-2} d^{-1} for the addition of 0,
432 5,000, 10,000, 20,000 and 50,000 mg L^{-1} sulfate, respectively, after a reaction time of
433 100 d. There was no significant difference in the TCE degradation efficiency for these

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434 batch experiments that added 0 to 50,000 mg L^{-1} sulfate. Therefore, sulfate
435 concentrations ranging from 5,000 to 50,000 mg L^{-1}, have no effect on TCE
436 degradation for the concentration of EcoClean of 5,000 mg L^{-1}. As shown in Figure
437 5b, sulfide concentrations, ranging from 0.7 to 1.0 mg L^{-1} were observed during the
438 experiments, which demonstrated that more sulfate reduction occurred in the
439 sulfate/EcoClean microcosms than in the microcosms where sulfate alone was added
440 (Figure 5a).

441 Figures 7b to 7e show the production of TCE degradation byproducts during the
442 experiments. As shown in Figure 7b, when sulfate was present in the system, the
443 concentrations of cis-DCE decreased significantly. Since the concentrations of other
444 TCE-degrading by-products were quite low and TCE degradation was dominated by
445 the concentration of EcoClean, the effect of sulfate addition on the removal of those
446 by-products was insignificant. It should be noted that sulfate played an important role
447 in the removal of cis-DCE and VC in the batches with no EcoClean addition (Figures
448 4b and 4e). This indicates that the presence of sulfate can ensure the complete
449 dechlorination of TCE when EcoClean is completely consumed. Accordingly, it is
450 feasible to use persulfate followed by a bioremediation reagent as a treatment train

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451 technology for TCE degradation. Notably, persulfate-releasing materials in the third
452 stage of the treatment train can be applied to further remove the by-products if the
453 accumulated concentrations of the residual by-products are high. In addition, at 100 d
454 reaction time, for 5,000 mg L^{-1} EcoClean support, the total number of soil bacteria
455 increases from 1.3×10^5 to 1.39×10^6, 6.49×10^6, 5.37×10^6, 3.45×10^6 and $1.43 \times$
456 10^6 CFU g^{-1} soil for the addition of 0, 5,000, 10,000, 20,000 and 50,000 mg L^{-1} sulfate,
457 respectively. Therefore, the presence of sulfate did not significantly affect the
458 bacterial growth during the experiments.
459
460 3.5 *The effect of a bioremediation reagent on DOC*
461 DOC in the microcosms increased from 80 mg L^{-1} to approximately 4,500, 8,000,
462 17,500 and 46,000 mg L^{-1} for the addition of 5,000, 10,000, 20,000 and 50,000 mg L^{-1}
463 EcoClean reagent, respectively. During the TCE biodegradation process, the DOC
464 decreased as the reaction time increased, which shows that carbon was consumed by
465 the indigenous bacteria during the anaerobic dechlorination. The pseudo-first order
466 rate constants for DOC utilization during 100 d of incubation were 2.2×10^{-2}, $1.9 \times$
467 10^{-2}, 1.0×10^{-2} and 3.0×10^{-3} d^{-1} for the addition of 5,000, 10,000, 20,000, and 50,000

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468 mg L^{-1} EcoClean, respectively. In order to determine the effect of sulfate on DOC
469 degradation, DOC in the batches where EcoClean at 5,000 mg L^{-1} was added to
470 different sulfate concentrations was also analyzed. The pseudo-first order rate
471 constants for DOC utilization were 2.2×10^{-2}, 2.0×10^{-2}, 1.6×10^{-2}, 1.7×10^{-2} and 1.3
472 $\times 10^{-2}$ d^{-1} for the addition of 0, 5,000, 10,000, 20,000, and 50,000 mg L^{-1} of sulfate,
473 respectively. The rate constant slightly decreased as the sulfate concentration
474 increased, which demonstrated that the presence of sulfate did not result in the
475 consumption of more DOC. Aulenta et al. [50] reported that the presence of sulfate
476 caused a slightly higher degradation rate for the added substrate, butyrate, and
477 decreased the rate of reductive dechlorination because there was rapid and
478 competitive utilization of the electron donors by sulfate-reducing populations.
479 However, no significant adverse impact on DOC consumption and TCE
480 dechlorination was seen in this study. Therefore, more studies of different sites are
481 required, in order to obtain more detailed information regarding the effect of sulfate
482 on DOC consumption.
483
484

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485 *3.6 RT-PCR and DGGE analysis*

486 Figure 8a shows the DGGE profiles for the PCR-amplified 16S rDNA during the
487 experiments. The green bars refer to the bacteria that appear in background soil. The
488 yellow bars refer to the bacteria that do not appear in the background soil. The red
489 bars refer to the bacteria that match the bacteria in background soil. Lanes 1 and 6-9
490 in Figure 6a show that some bands in the background soil (Lane 1) disappeared, while
491 some bacteria became significant, during 40 d of incubation. This demonstrates that
492 the addition of sulfate and EcoClean causes significant changes in the microbial
493 community. The bacterial community was also more abundant at Day 40 than that at
494 Day 55 (Lanes 2-5). Since TCE concentrations were low at Day 55, the microbial
495 activity may decrease, resulting in a reduction in bacterial abundance. In order to
496 better understand the relationships between microorganisms in different microcosms,
497 the UPGMA dendrogram of DGGE profiles was analyzed. Figure 8b shows that there
498 was little similarity between the microbial community in most samples, which
499 demonstrated that the addition of sulfate and EcoClean had a significant impact on the
500 indigenous bacterial community because of the utilization of different electron
501 acceptors by different predominant bacteria.

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502 Previous studies have reported that *Dehalococcoides* degrade TCE to VC and
503 ethane via reductive dechlorination [25, 51, 52]. The reductive dechlogenase, *vcr*A, of
504 *Dehalococcoides* sp. is responsible for the complete dechlorination of TCE to ethane
505 [53]. In this study, the RT-PCR technique was used to quantify the amounts of
506 *Dehalococcoides* and *vcr*A genes. The RT-PCR results show that *Dehalococcoides*
507 and *vcr*A genes were not detected at Day 0, while the number of *Dehalococcoides*
508 was 3,423 gene copies/g in the presence of 5,000 mg L^{-1} EcoClean reagent, at 40 d
509 reaction time. The existence of *Dehalococcoides* demonstrates the dechlorination
510 capability of the treatment train technology that is used in this study. The number of
511 *vcrA* genes was 1,105 gene copies/g in the presence of 5,000 mg L^{-1} EcoClean reagent
512 and sodium sulfate, which is higher than the figure of 498 gene copied/g in the
513 presence of 5,000 mg L^{-1} sodium sulfate, at 40 d reaction time. The addition of
514 EcoClean reagent contributed to the increase in *Dehalococcoides* and *vcrA* genes,
515 which are favorable for TCE degradation. The results of the RT-PCR analyses
516 demonstrate that TCE can be completely dechlorinated at the contaminated site,
517 because of the presence of dechlorinating bacteria and enzymes.
518

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519 *3.7 A Column experiment for the three-stage treatment train*

520 Using the results of previous batch experiments, a continuous column experiment
521 combined with chemical oxidation, anaerobic bioremediation and PRB treatment was
522 used to determine the effectiveness of the treatment train technology on TCE
523 degradation, for the first time. The concentrations of persulfate and EcoClean used
524 were both 10,000 mg L^{-1}, according to the results of the batch experiments. Figure 9
525 shows the variation in the TCE concentration in Columns 1 to 5. The average TCE
526 concentration in the effluent of Column 1 was approximately 45 mg L^{-1}, before
527 persulfate was added. When 10,000 mg L^{-1} persulfate is added to Column 1, the TCE
528 concentration decreased rapidly to 5 mg L^{-1} in 5 PV. The TCE concentration in the
529 effluent of Column 1 decreased from 5.0 to 4.1 and 3.0 mg L^{-1} in the effluents of
530 Columns 2 and 3, respectively. TCE was removed by in situ bacteria via reductive
531 dechlorination degradation, which was demonstrated by the detection of 0.2 mg L^{-1}
532 cis-DCE in the effluent of both Columns 2 and 3. After treatment by oxidant-releasing
533 materials that continuously released approximately 6,000 mg L^{-1} of persulfate in
534 Column 4, no TCE and its by-products were found in this section because of
535 persulfate oxidation. Our previous studies have demonstrated that oxidant-releasing

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536 materials were able to steadily release persulfate for at least 50 days [22, 23]. The PRB
537 system in the last stage of the treatment train plays an important role in preventing the
538 rebound of TCE and the accumulation of the TCE biodegrading by-products, which
539 ensures the success of site remediation. Tsai et al.[2] applied a three-stage treatment
540 train that was composed of surfactant flushing, permanganate oxidation, and aerobic
541 biodegradation to treat TCE-contaminated groundwater (40 mg L^{-1}). Although TCE
542 was removed effectively, their study was conducted by three separate static batch
543 experiments. The continuous column test used in this study can simulate the
544 application of the proposed treatment train in the field more appropriately. The results
545 for the column experiment demonstrate that 88% of TCE was removed in Column 1.
546 This then accumulated to 94% after the bioremediation treatment in Columns 2 and 3.
547 100% of the TCE was removed after PRB treatment in Column 4. No TCE or its
548 by-products were detected in the effluent of Column 5, which demonstrates that this
549 treatment train technology is very effective.

550 In this study, chemical oxidation and anaerobic biodegradation technologies
551 were employed simultaneously to remove TCE contamination in soil. Therefore, it is
552 important to determine the difference in the ORP between these two technologies. For

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553 example, a high ORP that is elevated by chemical oxidation can inhibit anaerobic
554 dechlorination, which generally requires a low ORP. In addition, a low ORP that is
555 caused by anaerobic bioremediation in Columns 2 and 3 can further affect the
556 oxidant-releasing material treatment in Column 4. An ORP of 10,000 mg L^{-1}
557 persulfate remained above 600 mV before it was injected to Column 1. The ORP of
558 the effluent in Column 1 decreased to 250 mV because there was a decrease in
559 persulfate concentration (30 mg L^{-1}). In Columns 2 and 3, the ORP decreased to lower
560 than -300 mV because of the decomposition of EcoClean by soil bacteria. This leads
561 to advantageous conditions for TCE dechlorination. ORP and persulfate
562 concentrations were around 500 mV and 4,000 mg L^{-1}, respectively, in the effluent of
563 Column 4, which contained oxidant-releasing materials. In Column 5, since persulfate
564 concentration decreased to 50 mg L^{-1}, a lower ORP (100 - 200 mV) was observed.
565 The column experiments show that there is no problem combining chemical oxidation
566 and anaerobic bioremediation in the treatment train technology for this study. The
567 results also show that ORP and persulfate concentration are important monitoring
568 parameters that determine the compatibility of each stage of the treatment train,
569 simply and rapidly, in the field.

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570 The initial DOC was around 9,000 mg L^{-1} when EcoClean reagent was added to
571 Columns 2 and 3. After 50 PV of reaction time, the DOC decreased to 4,000 mg L^{-1},
572 which demonstrated that the reagent was consumed by soil bacteria during the
573 anaerobic dechlorination of TCE. Column 1 had high persulfate concentration so the
574 total number of bacteria decreased from 1.23×10^5 to 6.7×10^4 CFU g^{-1} soil. In
575 Columns 2 and 3, when EcoClean reagent was added, the total number of bacteria
576 recovered and increased to 2.5×10^5 and 2.6×10^5 CFU g^{-1} soil, respectively. Therefore,
577 EcoClean reagent aids the growth of bacteria. In Column 5, the total number of soil
578 bacteria slightly decreased to 1.13×10^5 CFU g^{-1} soil due to the residual persulfate
579 from Column 4. The results show that the proposed treatment train did not cause
580 significant adverse effect on in situ bacteria.

581 It should be noted that the long-term effect of the treatment train on the bacterial
582 community is an important issue. In a column study, Richardson et al.[43] indicated that
583 the diversity of soil bacterial community was reduced immediately after persulfate
584 was injected. Although the microbial diversity increased after 30 days, it took 100 to
585 500 days for the recovery of phenanthrene-degrading bacterial groups. It is also
586 necessary to confirm that the substrate addition can help TCE-degrading bacteria

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587 maintain a good performance of TCE dechlorination during a long period of
588 remediation[54]. Therefore, the long-term effect of the treatment train operation on the
589 microbial diversity and specific TCE-degrading bacteria such as *Dehalococcoides*
590 need to be further evaluated in a future study.
591

592 **4. Conclusions**

593 Soil and ground contamination is complex and difficult to remediate using a
594 single technology. This study proposes a treatment train that comprises chemical
595 oxidation, anaerobic bioremediation and PRB treatment to remediate
596 TCE-contaminated groundwater. The results of the batch experiments show that a
597 high concentration of TCE was oxidized effectively by persulfate oxidation. A low pH
598 value and oxidative stress that is caused by the addition of persulfate may be the
599 factor that inhibits the number of bacteria in soil. The results of the microcosm study
600 demonstrate that the sulfate that is produced from persulfate oxidation could be
601 utilized by indigenous bacteria to ensure the complete dechlorination of TCE,
602 especially when no bioremediation reagent was present. The addition of EcoClean
603 significantly enhanced the dechlorination of TCE. Dechlorinating bacteria,

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604 *Dehalococcoides,* mainly contributed to the reductive dechlorination of TCE. The
605 results of this study show that 10,000 mg L^{-1} of persulfate and the bioremediation
606 reagent, and the PRB that can continuously release 6,000 mg L^{-1} of persulfate are
607 suggested to operate the treatment train. The designed three-stage treatment train used
608 low concentrations of persulfate coupled with the anaerobic bioremediation reagent to
609 remove TCE and its degrading by-products completely without significant adverse
610 effect on intrinsic microorganisms, which demonstrates that the proposed treatment
611 train is a feasible technology for the remediation of groundwater that is contaminated
612 with TCE. Future work should focus on the evaluation of the performance of TCE
613 removal by lower persulfate concentrations and the long-term effect of the treatment
614 train operation on the microbial community. The evaluation of the balance between
615 cost and operation time should also be further investigated prior to field application.
616

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716 Table 1. The components of the batch experiments.

Batch experiment	Treatment	Constituent
Persulfate oxidation	Persulfate oxidation alone	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
	Activated persulfate oxidation	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
	Sterilized control	TCE 50 mg L^{-1} + $HgCl_2$ 250 mg L^{-1} + aquifer sediment 10 g + DI water 50 mL
Anaerobic biodegradation	Anaerobic biodegradation with sulfate	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
	Anaerobic biodegradation with EcoClean	TCE 5 mg L^{-1} + EcoClean (0, 5,000, 10,000, 20,000 and 50,000 mg L^{-1}) + aquifer sediment 10 g + mineral medium 50 mL
	Anaerobic biodegradation with EcoClean + sulfate	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
	Sterilized control	TCE 5 mg L^{-1} + $HgCl_2$ 250 mg L^{-1} + aquifer sediment 10 g + mineral medium 50 mL

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718 **Figure captions**

719 Figure 1. The layout of the column experiments.

720

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

724

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

728

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

732

733 Figure 5. The production of sulfide in (a) sulfate addition and (b) EcoClean/sulfate
734 addition systems.

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735 Figure 6. The effect of EcoClean concentration on (a) TCE degradation; (b) cis-DCE
736 production; (c) trans-DCE production; (d) 1,1-DEC production and (e) VC
737 production.
738
739 Figure 7. The effect of sulfate concentration on (a) TCE degradation; (b) cis-DCE
740 production; (c) trans-DCE production; (d) 1,1-DEC production and (e) VC production,
741 in the presence of EcoClean.
742
743 Figure 8. The results of DGGE analysis for the batch anaerobic biodegradation
744 experiments: (a) the DGGE profiles for the PCR-amplified 16S rDNA and (b) the
745 similarity in the DGGE profiles.
746
747 Figure 9. The variation in TCE concentration in the column test for (a) Column 1 and
748 (b) Columns 2-4.

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Figure 1

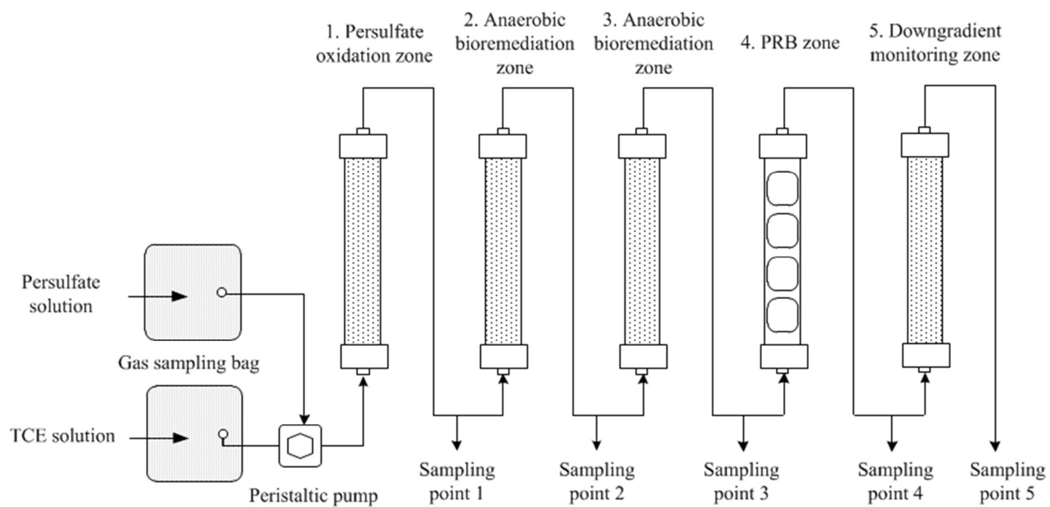

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Figure 2

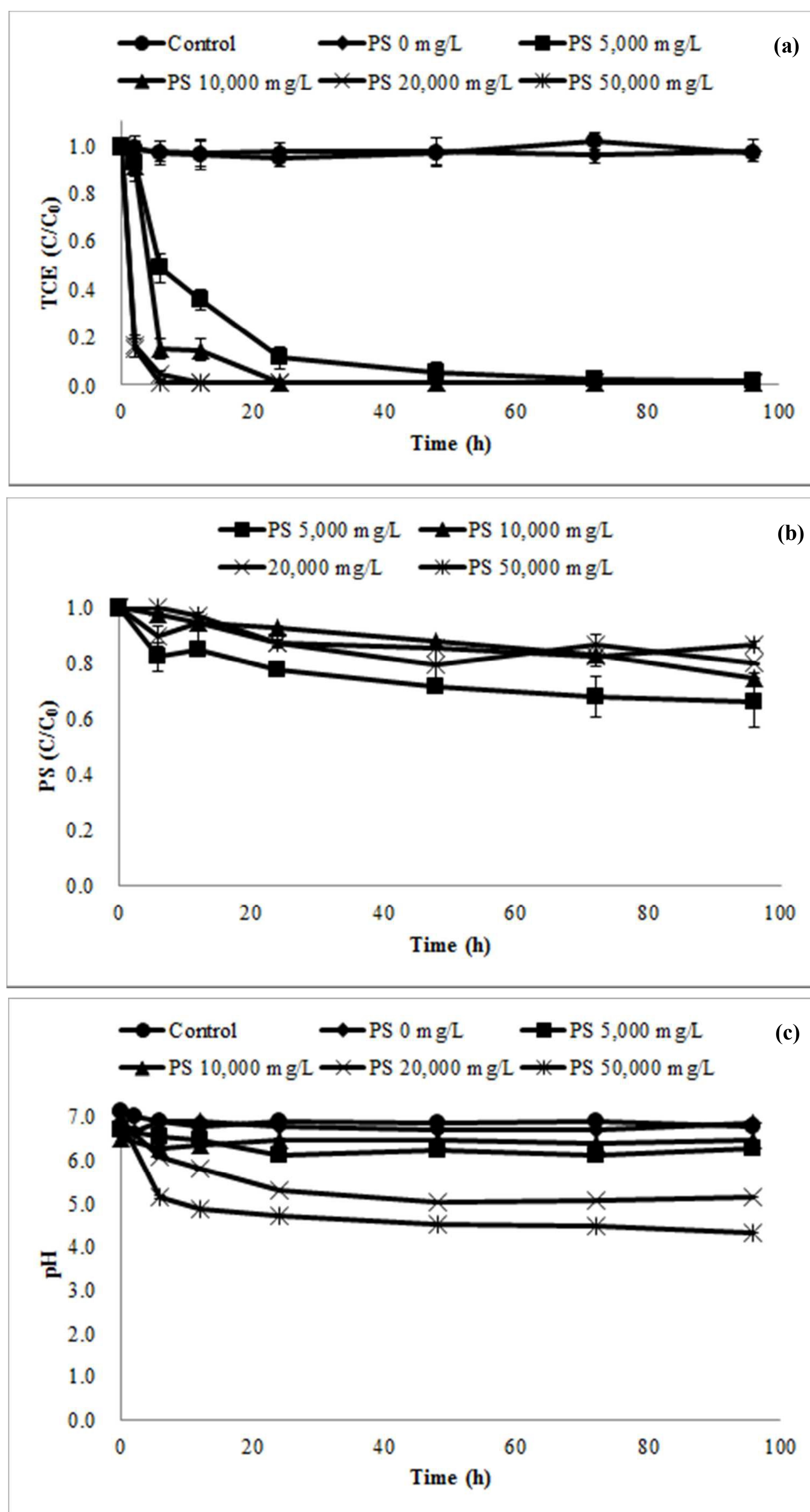

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Figure 3

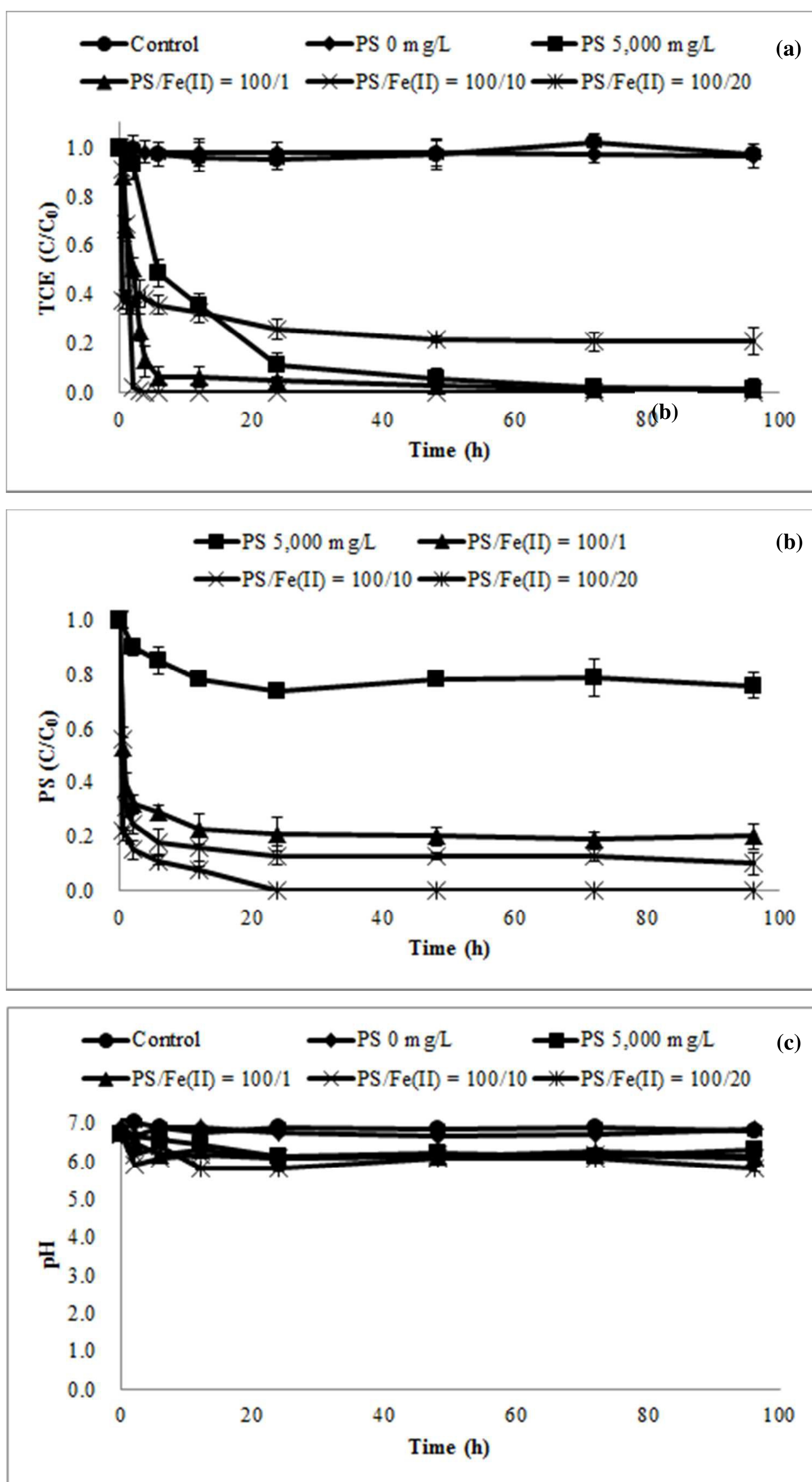

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Figure 4

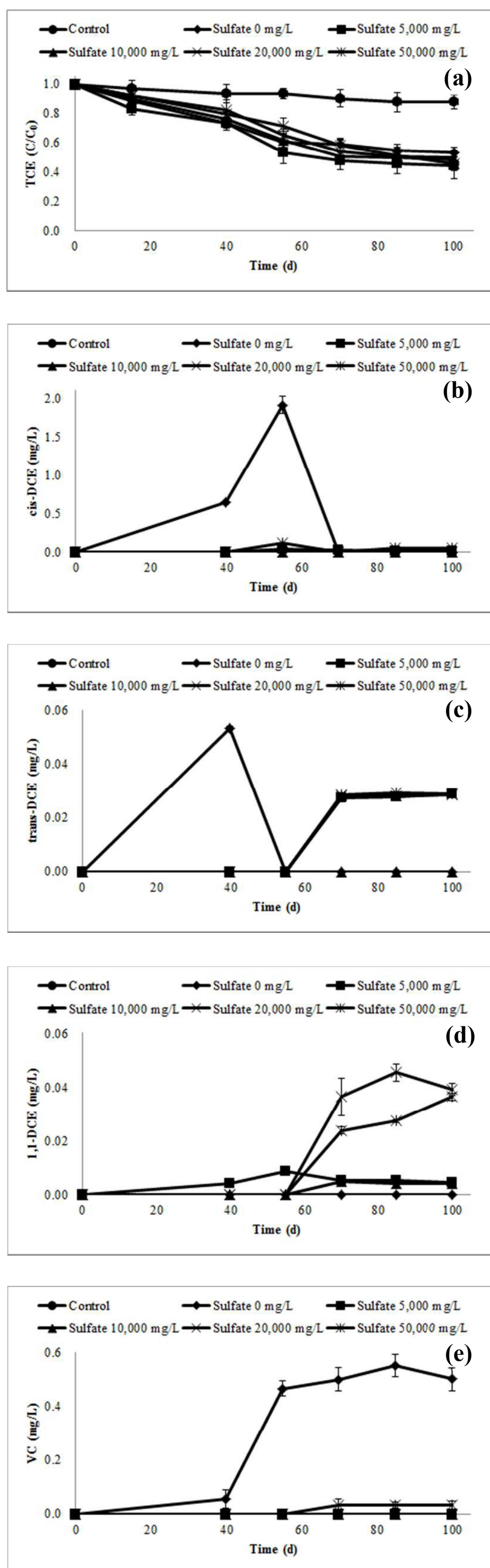

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Figure 5

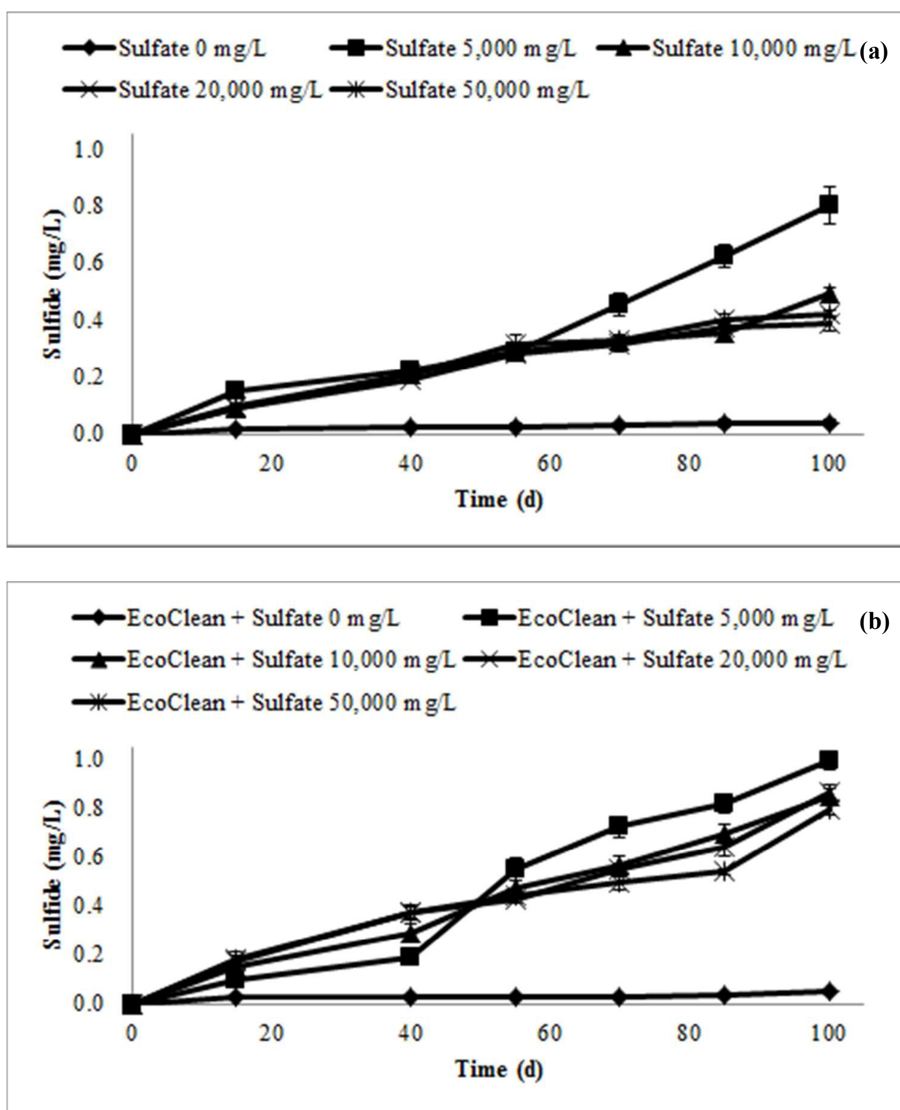

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Figure 6

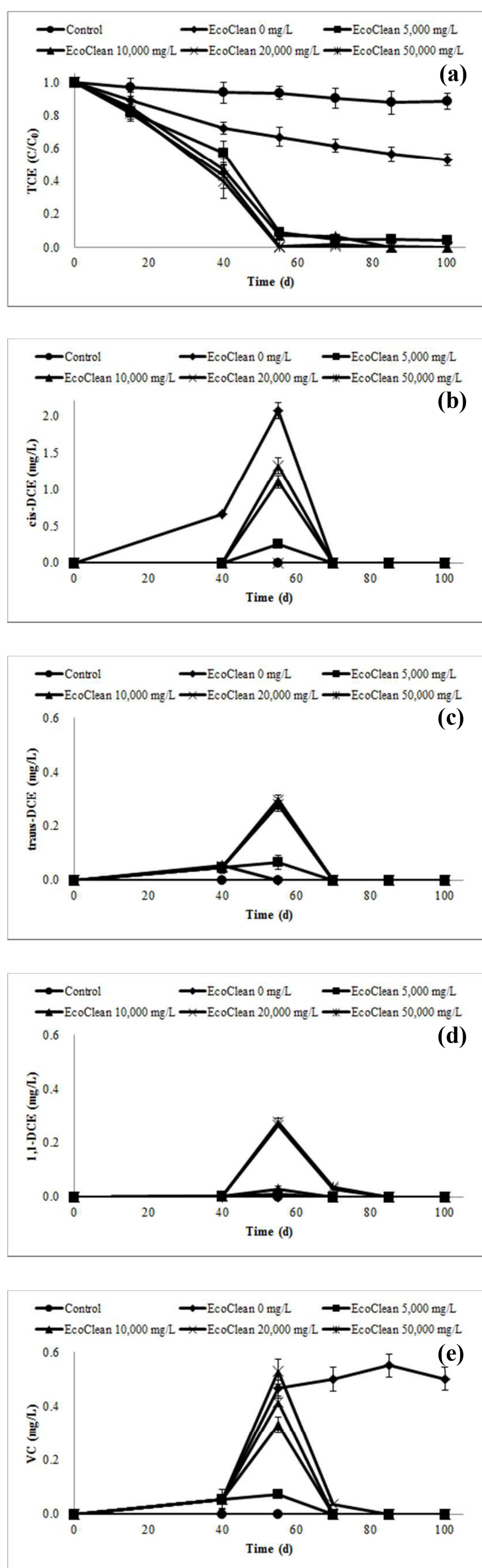

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Figure 7

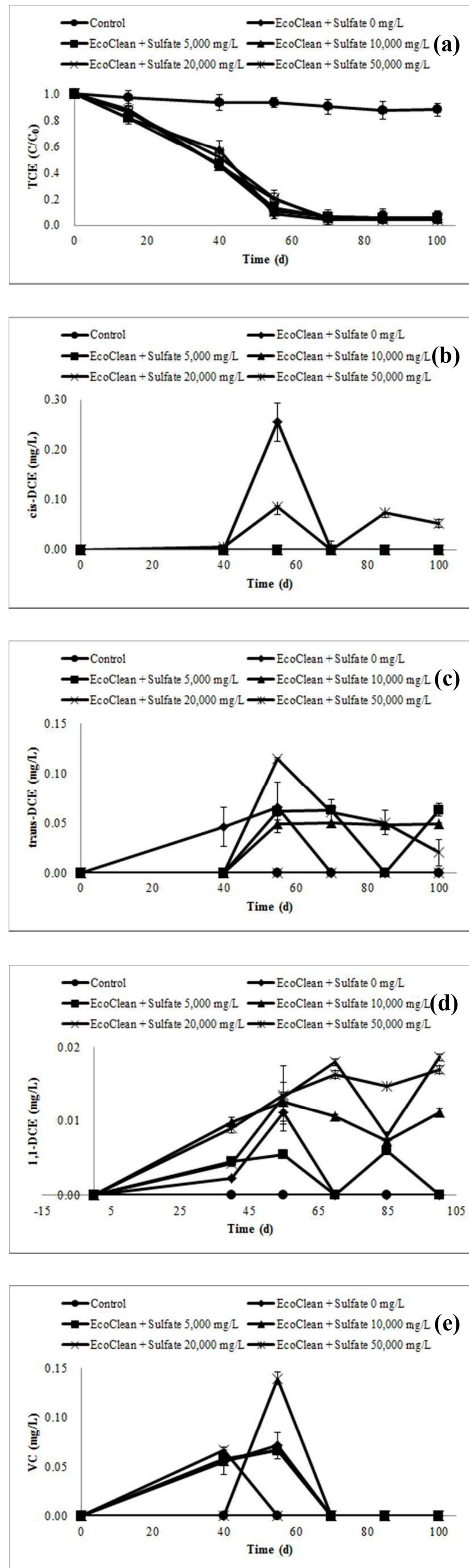

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Figure 8

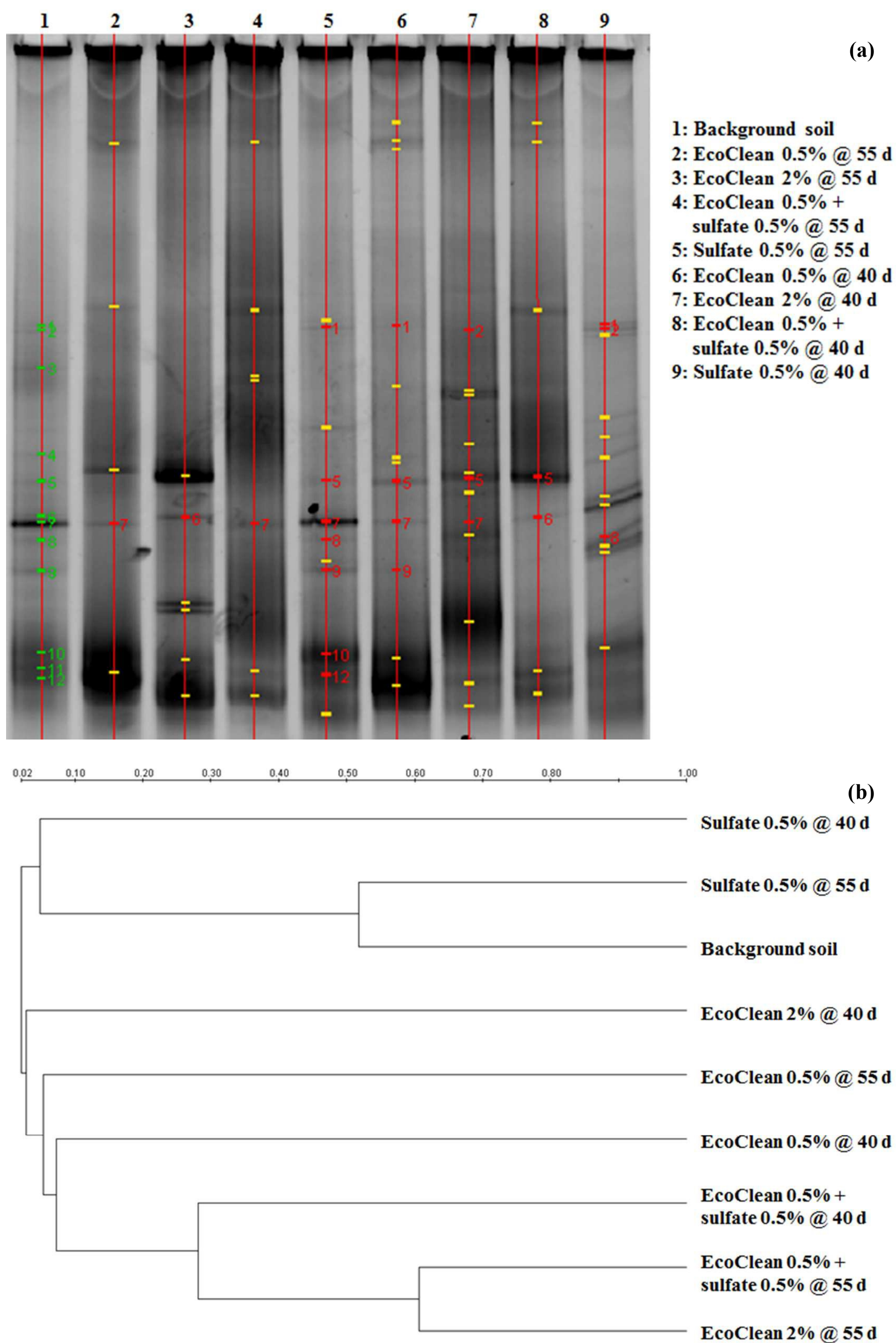

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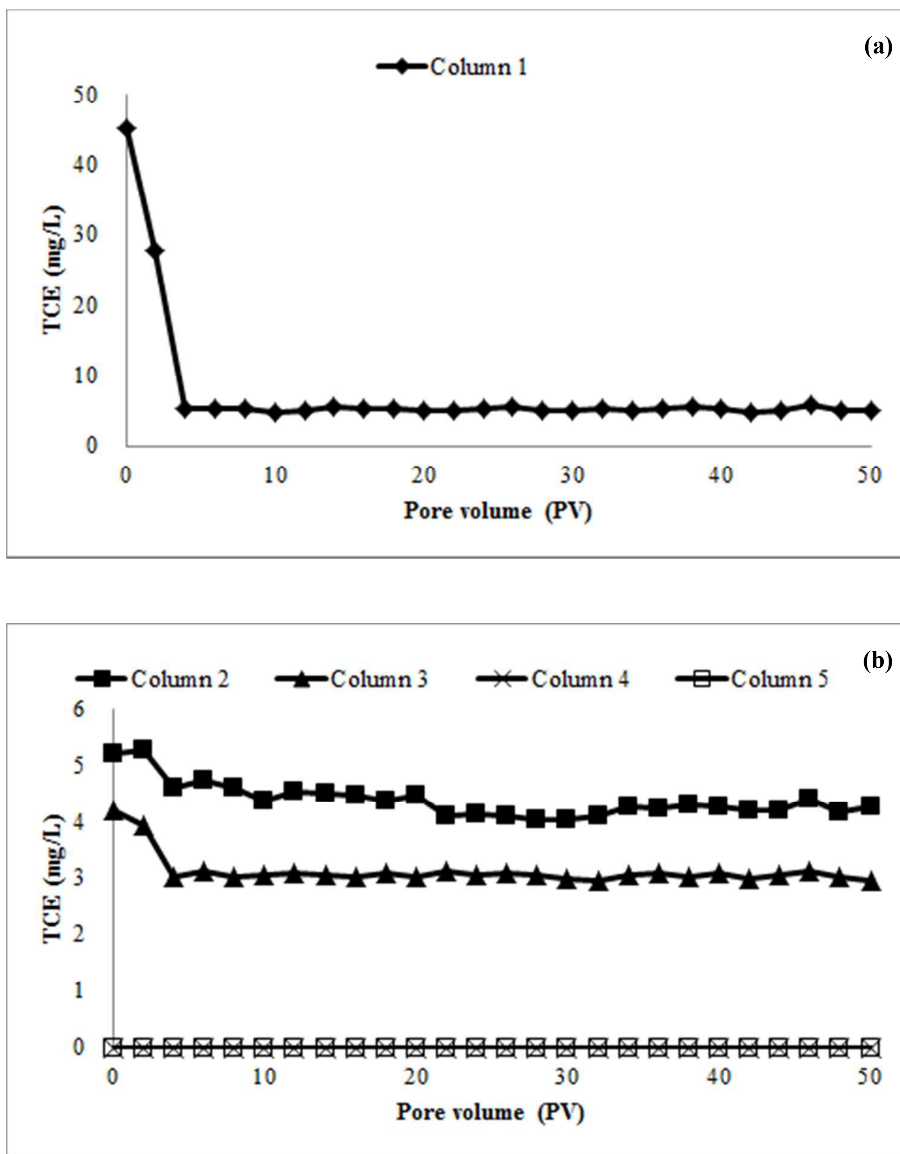
Figure 9



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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.