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## Abstract

13  
14 Enhanced reductive dehalogenation is an attractive treatment technology for in situ  
15 remediation of chlorinated solvent DNAPL source areas. Reductive dehalogenation is  
16 an acid-forming process with hydrochloric acid and also organic acids from fermenta-  
17 tion of the electron donors typically building up in the source zone during remedia-  
18 tion. This can lead to groundwater acidification thereby inhibiting the activity of deha-  
19 logenating microorganisms. Where the soils' natural buffering capacity is likely to be  
20 exceeded, the addition of an external source of alkalinity is needed to ensure sustained  
21 dehalogenation. To assist in the design of bioremediation systems, an abiotic geo-  
22 chemical model was developed to provide insight into the processes influencing the  
23 groundwater acidity as dehalogenation proceeds, and to predict the amount of bicar-  
24 bonate required to maintain the pH at a suitable level for dehalogenating bacteria (i.e.,  
25 > 6.5). The model accounts for the amount of chlorinated solvent degraded, site water  
26 chemistry, electron donor, alternative terminal electron-accepting processes, gas re-  
27 lease and soil mineralogy. While calcite and iron oxides were shown to be the key  
28 minerals influencing the soil's buffering capacity, for the extensive dehalogenation  
29 likely to occur in a DNAPL source zone, significant bicarbonate addition may be ne-  
30 cessary even in soils that are naturally well buffered. Results indicated that the bicar-  
31 bonate requirement strongly depends on the electron donor used and availability of  
32 competing electron acceptors (e.g., sulfate, iron(III)). Based on understanding gained  
33 from this model, a simplified model was developed for calculating a preliminary de-  
34 sign estimate of the bicarbonate addition required to control the pH for user-specified  
35 operating conditions.

36 *Keywords:* reductive dehalogenation, dechlorination, alkalinity, trichloroethene, bi-  
37 carbonate, electron donor, DNAPL, PHREEQC

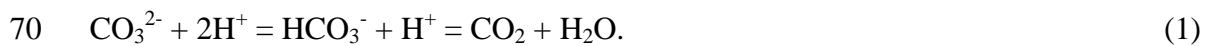
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## 38 1. Introduction

39 Chlorinated ethenes, such as tetrachloroethene (PCE) and trichloroethene  
40 (TCE), are among the most persistent and hazardous groundwater contaminants (Na-  
41 tional Research Council, 2004; Rivett et al., 2005). Enhanced reductive dehalogena-  
42 tion is widely used for the in situ remediation of chlorinated ethene plumes and is now  
43 recognized as a promising technology for DNAPL source areas (McCarty, 1997; Ellis  
44 et al., 2000; Major et al., 2002; Yang and McCarty, 2002; AFCEE, 2004). Here, bio-  
45 degradation is achieved by stimulating the activity of dehalogenating bacteria – e.g.,  
46 *Dehalococcoides* (Major et al., 2002; Löffler and Edwards, 2006), *Sulfurospirillum*  
47 *multivorans* (Neumann, 1994; Amos et al., 2007; Amos et al., 2008), *Dehalobacter*  
48 *restrictus* (Schumacher and Holliger, 1996) – through the addition of an electron do-  
49 nor. Recent studies have indicated that remediation in the proximity of the source  
50 zone, rather than dilute plume dehalogenation, results in more efficient degradation  
51 due to enhanced rates of solvent dissolution and thus reduction in the longevity of the  
52 DNAPL plume (Yang and McCarty, 2000; Amos et al., 2008).

53 Although increased understanding of dehalogenating bacteria and suitable  
54 electron donors has led to more rapid dehalogenation rates (Carr and Hughes, 1998;  
55 Aulenta et al., 2006), complete dehalogenation to ethene is still often hindered at  
56 many sites due to, for example, inadequate electron donor supply (Yang and McCarty,  
57 2002; Aulenta et al., 2006; Löffler and Edwards, 2006; Aulenta et al., 2007), high  
58 concentrations of alternative electron acceptors (e.g., sulfate) (Hoelen and Reinhard,  
59 2004; Heimann et al., 2005; Aulenta et al., 2006), insufficient contact time (Da Silva  
60 et al., 2006), absence of suitable consortia of dehalogenating bacteria (Löffler and  
61 Edwards, 2006; Amos et al., 2007) and development of low groundwater pH (Cope

62 and Hughes, 2001; Adamson et al., 2004; McCarty et al., 2007). Reductive dehaloge-  
63 nation occurs in a step-wise manner converting PCE to TCE to dichloroethene (DCE)  
64 to vinyl chloride (VC), and finally to ethene. Each step involves the removal of one  
65 chlorine atom from the chlorinated ethene molecule, giving rise to hydrochloric acid  
66 (HCl) production. The combination of this strong acid and the build-up of organic  
67 acids formed during electron donor fermentation can result in groundwater acidifica-  
68 tion (Adamson et al., 2004; AFCEE, 2004; Chu et al., 2004; Amos et al., 2008). The  
69 groundwater pH is typically strongly controlled by the dissolved carbonate equilibria,



71 The acid formed during dehalogenation reacts with bicarbonate ( $\text{HCO}_3^-$ ) to produce  
72 carbon dioxide ( $\text{CO}_2$ ). In aquifers,  $\text{CO}_2$  is not readily released to the atmosphere, and  
73 the increase in dissolved  $\text{CO}_2$  coupled with the decrease in  $\text{HCO}_3^-$  depresses the pH  
74 further. This is evident from the  $\text{HCO}_3^-/\text{CO}_2$  equilibrium expression:

$$\frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2]} = K = 10^{-6.3}, \quad (2)$$

75 where  $K$  is the equilibrium constant and the bracketed quantities denote molar  
76 aqueous concentrations.

77 Laboratory studies have demonstrated that the optimal pH range for dehaloge-  
78 nating microorganisms is 6.8 – 7.8 (Middeldorp et al., 1999; Cope and Hughes, 2001;  
79 AFCEE, 2004) and, correspondingly, that low pH leads to reduced dehalogenation  
80 rates (Cirpka et al., 1999; Adamson et al., 2004). Acidic conditions inhibit, in particu-  
81 lar, the dehalogenation of the lesser chlorinated ethenes (Christ et al., 2005). Where  
82 pH drops are expected or observed, an alkalinity source such as sodium or potassium

83 bicarbonate can be added to raise and/or neutralize the pH (AFCEE, 2004; Payne et  
84 al., 2006). Other buffers such as sodium carbonate or hydroxide tend to provide unst-  
85 able pH control, while lime (CaO) addition is likely to lead to calcite (CaCO<sub>3</sub>) precipi-  
86 tation and subsequent aquifer clogging (McCarty et al., 2007). Bicarbonate addition  
87 offsets the impact of the higher dissolved CO<sub>2</sub> concentrations produced from dehalo-  
88 genation. With recent developments (e.g., the availability of increasingly effective  
89 bacterial consortia, electron donors, injection strategies, etc.) allowing for more com-  
90 plete and rapid dehalogenation, more acidity is generated and thus there is an increas-  
91 ing need for pH control strategies. Furthermore, acidification is more likely during  
92 DNAPL source area bioremediation due to the higher mass of chlorinated ethenes  
93 dehalogenated compared with dilute plume bioremediation.

94         The two main issues associated with the design of pH control strategies are (1)  
95 the amount of bicarbonate addition needed as dehalogenation proceeds, and (2) how  
96 best to deliver this bicarbonate to the DNAPL source area. This study focuses on the  
97 first issue. McCarty et al. (2007) calculated the amount of reductive dehalogenation  
98 likely to occur prior to pH inhibition for a range of electron donors and initial bicar-  
99 bonate alkalinities. While they demonstrated that bicarbonate addition is likely re-  
100 quired for effective dehalogenation in source areas, the study raised a number of ques-  
101 tions including the influence of mineralogy, competing terminal electron-accepting  
102 processes (TEAPs) and gas release on the acidity generated and the subsequent  
103 amount of bicarbonate required to maintain the pH at a suitable level for dehalogenat-  
104 ing bacteria. Quantitative responses to these questions would clearly benefit detailed  
105 bioremediation system design.

106 This paper presents an abiotic geochemical model to address these issues. The  
107 model is implemented through the geochemical program PHREEQC version 2.15  
108 (Parkhurst and Appelo, 1999). The model is first described and simulation results for  
109 conditions pertinent to a typical remediation site are presented. The model accounts  
110 for the amount of dehalogenation, site water chemistry, electron donor, potential gas  
111 release, use of acetate as an electron donor, competing TEAPs and the precipitation  
112 and dissolution kinetics of common minerals. Following this, the paper explores the  
113 main factors influencing the bicarbonate requirements: (1) mineralogy, (2) electron  
114 donor, (3) minimum design pH, (4) acetate oxidization, and (5) competing TEAPs.  
115 Based on insight gained from these analyses, a simplified model is presented that may  
116 be used to calculate a preliminary estimate of the bicarbonate addition required once  
117 the minimum design pH is reached.

## 118 **2. Process understanding and model development**

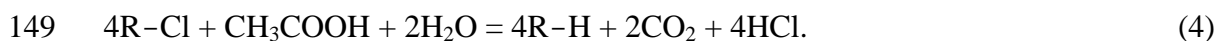
119 Enhanced reductive dehalogenation is a complex, microbially mediated  
120 process. Rather than simulating the suite of biological reactions influencing dehaloge-  
121 nation, the objective here was to develop an abiotic geochemical model focused on  
122 predicting the acidity generated during dehalogenation and the bicarbonate required  
123 for pH control. The main processes influencing the groundwater pH as dehalogenation  
124 proceeds are presented in Fig. 1. pH control is achieved by balancing the acidity and  
125 alkalinity perturbations using bicarbonate amendment. In this section, the various  
126 processes given in Fig. 1 are described.

127 Acidity is generated directly from dehalogenation (i.e., HCl) and from the by-  
128 products of electron donor fermentation. The model assumes that the remediation  
129 scheme will degrade a given amount of chlorinated ethenes according to:



131 By simulating the reduction of a generic chlorinated ethene compound (R-Cl), the  
132 results can be interpreted regardless of which chlorinated ethene is actually reduced.  
133 That is, Cl production is used to quantify the amount of dehalogenation. While the H<sub>2</sub>  
134 required for dehalogenation in (3) can be directly injected into the aquifer, more often  
135 an organic, fermentable electron donor is used. The acidity generated from fermenta-  
136 tion depends on the specific electron donor used, with each producing different  
137 amounts of acetate and carbonate species (McCarty et al., 2007). Fermentation reac-  
138 tions for common donors based on standard biochemical pathways are given in Table  
139 1. While acetate and carbonate species increase acidity, the presence of sodium asso-  
140 ciated with an electron donor (e.g., sodium lactate and formate) reduces this acidity  
141 because bicarbonate is formed upon fermentation rather than carbon dioxide (McCar-  
142 ty et al., 2007) (Fig. 1).

143 Acetate generated from electron donor fermentation can also serve as an elec-  
144 tron donor for conversion of PCE and TCE to DCE, but not directly, if at all, in the  
145 conversion of DCE and VC (Dolfing and Tiedje, 1991; Krumholz et al., 1996; Sharma  
146 and McCarty, 1996; Löffler et al., 2000; Sung et al., 2003; Lee et al., 2007). With R-  
147 Cl representing PCE or TCE, the dehalogenation reaction with acetate as the electron  
148 donor is:



150 This process not only reduces the overall electron donor requirements and concentra-  
151 tion of acetic acid, but also leads to the production of CO<sub>2</sub>, thus shifting the carbonate  
152 equilibrium in (2). There is also some evidence that acetate might be fermented to H<sub>2</sub>

153 and CO<sub>2</sub>, and the H<sub>2</sub> produced might then be used for dehalogenation of DCE and VC  
154 (He et al., 2002). A parameter,  $p$ , is used in the model to specify the fraction of acetate  
155 produced from donor fermentation that is subsequently used as an electron donor.

156 Not all the H<sub>2</sub> and acetate produced from fermentation are used for dehaloge-  
157 nation as dehalogenating bacteria must compete for these electron donors with other  
158 microbial populations such as denitrifiers, iron and sulfate reducers and methanogens  
159 (Table 2). In addition to these TEAPs increasing the amount of electron donor fer-  
160 mented, and thus the acidity generated for a given level of dehalogenation, each  
161 TEAP adds a different amount of alkalinity per mol of H<sub>2</sub> consumed (Table 2). In the  
162 presence of multiple electron acceptors, species are generally reduced in order of  
163 thermodynamic preference: oxygen reduction > nitrate reduction > iron(III) reduction  
164 > dehalogenation > sulfate reduction > methanogenesis (Löffler et al., 1999; Curtis,  
165 2003). While this sequence, and thus the fraction of H<sub>2</sub> directed to dehalogenation,  
166 may be predicted based on thermodynamic considerations (Gibbs free energy of reac-  
167 tion,  $\Delta G_r^\circ$ ), competition also depends on microbial populations and specific field  
168 conditions (Dolfing and Janssen, 1994; Jakobsen and Postma, 1999; Löffler et al.,  
169 1999; Curtis, 2003). Methanogenesis is not included in the model as this TEAP is  
170 assumed to be inhibited by the low H<sub>2</sub> concentrations and high chlorinated solvent  
171 concentrations in the source zone (Yang and McCarty, 2000). Furthermore, oxygen  
172 and nitrate reduction are not considered as these electron acceptors must be reduced  
173 before dehalogenating conditions can be induced (AFCEE, 2004). Sulfate and  
174 iron(III) reduction however often occur concomitantly with dehalogenation and thus  
175 these electron acceptors compete for H<sub>2</sub> (AFCEE, 2004; Heimann et al., 2005; Aulen-  
176 ta et al., 2007). As the proportion of H<sub>2</sub> used for dehalogenation is not known a priori  
177 due to the complexity of the microbial processes, the model assumes that at least a



178 fraction ( $f_{min}$ ) of the H<sub>2</sub> generated by electron donor fermentation is used for dehalo-  
179 genation with the remainder ( $1 - f_{min}$ ) used for iron(III) and sulfate reduction (Fig. 1).

180 For arbitrary  $f$ , the dehalogenation reaction (3) and fermentation reaction for  
181 each electron donor can be combined giving an overall stoichiometry for dehalogena-  
182 tion (Table 1). Our modeling approach is to follow this overall dehalogenation reac-  
183 tion as it progresses in reaction steps. The sequence of calculations performed at each  
184 step is outlined in Fig. 2. In the simulations presented, 40 mM of chlorinated ethene  
185 compound (R-Cl) are assumed to degrade over 100 d (the residence time for water to  
186 flow through a hypothesized DNAPL source zone). This residence time is divided  
187 equally into 500 reaction steps. Although complete dehalogenation of TCE at its solu-  
188 bility limit (7.6 mM) to ethene corresponds to only 22.8 mM of chlorinated ethene  
189 compound degraded, it is envisaged that with effective remediation scheme design  
190 (i.e., design that leads to favorable dehalogenating conditions in the source zone in-  
191 cluding the presence of a suitable electron donor and consortia of dehalogenating bac-  
192 teria, and neutral pH conditions), the amount of dehalogenation occurring may extend  
193 beyond this with more PCE and TCE dissolving into solution as transformation to  
194 lesser chlorinated compounds proceeds.

195 For each reaction step,  $f$  is first calculated based on the availability of iron(III)  
196 and sulfate.  $f$  is set to an assumed minimum value ( $f_{min}$ ) when these electron acceptors  
197 are in excess (i.e., 0.2 (AFCEE, 2004)). As iron(III) and sulfate become limited,  $f$  is  
198 calculated such that only the H<sub>2</sub> required to reduce the iron(III) and sulfate available  
199 will be produced in the overall dehalogenation reaction. It follows that when sulfate  
200 and iron oxides are depleted,  $f$  is set to unity and all H<sub>2</sub> generated from fermentation  
201 goes to dehalogenation. With  $f$  calculated, 40/500 mM of R-Cl (total mM of R-Cl to

202 degrade/number of time steps) then reacts according to the overall dehalogenation and  
203 fermentation reaction for the selected electron donor (Table 1). Following this,  
204 PHREEQC equilibrates the solution and, in doing so, the nonchlorinated electron ac-  
205 ceptors consume the surplus H<sub>2</sub> produced in the reaction. The sequence by which  
206 iron(III) and sulfate are consumed when the solution is equilibrated is based on ther-  
207 modynamics and therefore at each step the available iron(III) is reduced in preference  
208 to sulfate.

209 As the solution is speciated, minerals are allowed to dissolve and/or precipi-  
210 tate. Carbonate minerals, in particular calcite, are typically the main source of natural  
211 alkalinity. Other minerals such as silicates may provide important buffering capacity  
212 (Appelo and Postma, 2005); however, unlike carbonate minerals that dissolve rapidly,  
213 these minerals are typically slow to equilibrate and thus their buffering capacity is  
214 strongly kinetically-controlled. The dissolution and reduction of iron oxides (e.g.,  
215 goethite [FeOOH], ferrihydrite [Fe(OH)<sub>3</sub>]) also adds alkalinity whilst consuming H<sub>2</sub>  
216 (Table 2, Fig. 1). Although the dissolution and subsequent reduction of iron oxides is  
217 also kinetically controlled, the process is often enhanced by iron-reducing bacteria  
218 (Maurer and Rittmann, 2004). Finally, iron sulfides, in particular acid volatile sulfide  
219 (FeS), may precipitate rapidly following sulfate and iron(III) reduction (Rickard,  
220 1995). The direct precipitation of iron sulfide adds acidity according to:



222 However, when the overall reaction for iron sulfide precipitation, including sulfate  
223 and iron(III) reduction is considered:



225 it is evident that the overall reduction and precipitation process adds alkalinity to the  
226 solution. Although cation exchange can also influence sediment's natural buffering  
227 capacity, simulations indicate that these effects are only likely to be significant when  
228 the pH drops below approximately 4.5. Thus this process is not included in the model.

229 As the solution is speciated, a gas phase is also allowed to form and gas is re-  
230 leased if the sum of the partial pressures of all the gases present ( $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2$ ,  $\text{H}_2\text{O}$ ,  
231  $\text{H}_2$ ,  $\text{H}_2\text{S}$ ,  $\text{O}_2$ ) exceeds a given total pressure. This total pressure corresponds to a given  
232 depth below the water table, assuming that the water flow is predominantly horizon-  
233 tal. The release of  $\text{CO}_2(\text{g})$  influences the groundwater acidity as indicated in (2).

234 Finally, upon speciation of the solution, the pH is calculated (Fig. 2). Once the  
235 pH decreases to the microbial inhibition level ( $\text{pH} = 6.5$  in these simulations), itera-  
236 tions are performed such that sufficient bicarbonate is added to maintain this pH (i.e.,  
237 in Fig. 1 the acidity and alkalinity added must be equal). This procedure (Fig. 2) is  
238 repeated until the total number of steps is reached and thus the required amount of R-  
239 Cl is degraded. In the simulations presented in this paper, sodium bicarbonate ( $\text{NaH-}$   
240  $\text{CO}_3$ ) is used as the bicarbonate source; however the results are insensitive to whether  
241  $\text{NaHCO}_3$  or another bicarbonate salt such as potassium bicarbonate ( $\text{KHCO}_3$ ) is add-  
242 ed. Addition of calcium bicarbonate however is not recommended as it will likely lead  
243 to calcite precipitation and aquifer clogging in the remediation zone.

### 244 **3. Model setup for base conditions**

245 The model was first setup to simulate conditions pertinent to a typical remedi-  
246 ation site. Sensitivity analyses were then performed on this base case. The operating  
247 and design parameters used are as follows:

- 248 • Initial solution composition is specified using typical values of the major con-  
 249 stituents at contaminated chlorinated solvent sites (Table 3) (AFCEE, 2004).
- 250 • 40 mM of chlorinated ethene compound (R-Cl) degrades over 100 d.
- 251 • The inhibition level for bacteria and therefore minimum design pH = 6.5.
- 252 • Linoleic acid is used as the electron donor. This is a typical major component  
 253 of water insoluble electron donors such as emulsified vegetable oil which are  
 254 increasingly being used due to their slow controlled release rate (AFCEE,  
 255 2004; Long and Borden, 2006).
- 256 • Acetate is not used as an electron donor ( $p = 0$ ).
- 257 •  $f_{\min} = 0.2$ . Design factors for  $f$  commonly used to calculate the quantity of elec-  
 258 tron donor required for bioremediation are of the order of 0.2 to 0.5 (AFCEE,  
 259 2004).
- 260 • An excess of calcite ( $\text{CaCO}_3$ ) is present and in equilibrium with the solution,  
 261 i.e., saturation index (SI) = 0.
- 262 • Mass fraction of iron oxides in the soil is 7.5 wt % (3.4 mol kg of water<sup>-1</sup>).  
 263 This mass fraction is based on the mineralogy at a contaminated chlorinated  
 264 solvent site currently undergoing enhanced bioremediation. The dissolution of  
 265 iron oxides is controlled by the rate ( $R$ , mol m<sup>-3</sup> s<sup>-1</sup>):

$$266 \quad R = k \frac{A_0}{V} \left( \frac{m}{m_0} \right) [H^+]^{0.45}, \quad (6)$$

267 where  $k$  (mol m<sup>-2</sup> s<sup>-1</sup>) is the rate constant,  $A_0$  (m<sup>2</sup>) is the initial surface area of  
 268 iron oxides,  $V$  (m<sup>3</sup>) is the solution volume,  $m_0$  (mol) is the initial moles and  $m$   
 269 (mol) is the undissolved moles of iron oxides (Appelo and Postma, 2005). The  
 270 specific mineral surface area = 55 m<sup>2</sup> g<sup>-1</sup> (Roden, 2006) and  $k$  is 10<sup>-10.2</sup> mol m<sup>-2</sup>  
 271 s<sup>-1</sup>. The latter value assumes that 10% of the iron oxides are ferrihydrite (fresh-

272 ly precipitated) with the remainder being stable, well-crystallized goethite  
273 (Appelo and Postma, 2005).

- 274 • Iron sulfide is initially absent but it is allowed to precipitate if the solution be-  
275 comes oversaturated ( $SI > 0$ ).
- 276 • A gas phase is allowed to form once the sum of the partial pressure of all gases  
277 present exceeds 1.5 atm. This total pressure is equivalent to a location approx-  
278 imately 5 m below the watertable. The initial partial pressure for all the gases  
279 is negligible except  $N_2$  for which the partial pressure is set at 0.79 atm (Amos  
280 and Mayer, 2006), and  $CO_2$  for which the partial pressure is fixed by specifica-  
281 tion of the initial solution alkalinity and pH (Table 3).

## 282 **4. Results**

### 283 **4.1 Base conditions**

284 For the base conditions it is predicted that pH control is necessary when more  
285 than ~4.5 mM of chlorinated ethene equivalents (Cl) are produced from dehalogena-  
286 tion (Fig. 3a). Although there is an excess of calcite present, the results indicate that  
287 its buffering capacity is not sufficient to maintain the pH above 6.5. The dissolution of  
288 calcite is limited by its solubility rather than kinetic constraints, and only 0.037 mol of  
289 calcite are predicted to dissolve for 40 mM of dehalogenation (— in Fig. 3e). Al-  
290 though calcite is not able to supply sufficient pH control for sustained dehalogenation,  
291 its buffering capacity is still important as simulations indicate that if calcite is initially  
292 absent, the pH reaches 6.5 after only 0.8 mM of dehalogenation compared to after 3.6  
293 mM of dehalogenation if calcite is present (Fig. 3a). It should be noted that for the  
294 simulation without bicarbonate addition, once the pH drops below 6.5 the results are

295 theoretical because as the acidity increases the bacteria would become inhibited and  
296 thus, in reality, dehalogenation would stall.

297 For the conditions simulated, the total bicarbonate required to maintain the pH  
298 at 6.5 along with dehalogenation of 40 mM of chlorinated ethene equivalents is ~197  
299 mM (Fig. 3b). This total requirement is the sum of the initial solution alkalinity plus  
300 the bicarbonate that needs to be added. The initial alkalinity affects the extent of deha-  
301 logenation likely to occur prior to microbial inhibition, however, once the design pH  
302 is reached, the total bicarbonate required to maintain that pH as dehalogenation  
303 proceeds is the same. With  $f_{min} = 0.2$ , sulfate is depleted after 10.9 mM of dehalogena-  
304 tion (Fig. 3c). In contrast, iron(III) reduction (rate controlled) and iron sulfide precipi-  
305 tation continue as dehalogenation proceeds (Fig. 3e). Once sulfate is depleted,  $f$  is  
306 automatically adjusted to ~0.88 such that the  $H_2$  directed away from dehalogenation  
307 matches that required for iron(III) reduction, the sole remaining nonchlorinated TEAP  
308 when methanogenesis is inhibited by high PCE or TCE concentrations. The availabili-  
309 ty of iron(III) and therefore the adjusted  $f$ , depends on the iron oxide dissolution rate  
310 (6). When sulfate is present, the bicarbonate addition required to match the acidity  
311 generated is ~7.5 mM per mM of dehalogenation. This requirement reduces to ~4.9  
312 mM per mM of dehalogenation after sulfate has been depleted (Fig. 3b). The decrease  
313 in the bicarbonate requirement is primarily due to less acetic acid produced per mM of  
314 dehalogenation as  $f$  increases (Fig. 3d). The effects however are complicated because  
315 sulfate and iron(III) reduction and iron sulfide precipitation also influence the alkalini-  
316 ty (Table 2 and Equation 5). These effects are discussed further in Section 4.6.

317 Due to the common ion effect, when bicarbonate is added the build-up of car-  
318 bonate species leads to a net precipitation of calcite rather than dissolution (Fig. 3e).

319 The calcite that dissolves as the pH decreases from 7 to 6.5 rapidly re-precipitates  
320 upon bicarbonate addition. However, calcite precipitation is not significant as dehalo-  
321 genation and bicarbonate addition continue. This result reveals that the amount of  
322 bicarbonate required does not depend on the amount of calcite present.

323 The model predicts that, for the conditions simulated, the build-up of dissolved  
324 CO<sub>2</sub> accompanying dehalogenation and bicarbonate addition leads to gas bubble for-  
325 mation after 9.9 mM of dehalogenation (Fig. 3f). This is when the partial pressure of  
326 all the gases sums to 1.5 atm. Whilst N<sub>2</sub> is the dominant species when the gas phase  
327 forms (initial partial pressure = 0.79), the gas composition changes as dehalogenation  
328 proceeds with CO<sub>2</sub>(g) becoming the major component. Due to the shift in the carbo-  
329 nate equilibria (2) as CO<sub>2</sub>(g) is released, the bicarbonate requirement is reduced from  
330 ~4.9 mM when the gas phase initially forms to ~2.6 mM per mM of dehalogenation as  
331 dehalogenation and CO<sub>2</sub>(g) release continues (— in Fig. 3b). If a gas phase is not  
332 permitted to form, the amount of bicarbonate required per additional mol of dehaloge-  
333 nation is constant (– – in Fig. 3b).

#### 334 **4.2 Influence of mineralogy**

335 Dissolution, reduction and precipitation kinetics for common crystalline min-  
336 erals (calcite, iron oxides, gypsum, anorthite, K-feldspar, albite, chlorite and illite)  
337 were included in the model to identify minerals likely to influence the sediment buf-  
338 fering capacity over the timescale for groundwater to flow through the treatment zone  
339 (i.e., 100 d). For all minerals except gypsum, the rate expressions implemented and  
340 rate constants adopted were based on Appelo and Postma (2005). For gypsum, the rate  
341 expression of Singh and Bajwa (1990) was employed. The simulation revealed that  
342 calcite and gypsum dissolution, and iron oxide reduction are the main crystalline min-

343 eral processes likely to significantly influence the soil's natural buffering capacity  
344 over this timescale. The amounts of each mineral that dissolved for 40 mM of dehalo-  
345 genation with no bicarbonate added are provided in Table 4. The initial amounts  
346 present are based on the mineralogy at a contaminated chlorinated solvent site cur-  
347 rently undergoing enhanced bioremediation. Gypsum was absent in the contaminated  
348 layer at this particular site, but included here to examine its potential for dissolution.  
349 Simulations demonstrated that iron oxide reduction and dissolution is strongly rate  
350 controlled, while calcite and gypsum dissolution is of the order of hours and therefore  
351 these minerals can be considered to be in equilibrium ( $SI = 0$ ). Sulfate containing  
352 minerals such as gypsum can influence the bicarbonate requirement due to the buffer-  
353 ing effects of sulfate reduction. The influence of the sulfate availability is examined in  
354 Section 4.6.1 and the influence of the iron oxide reduction rate is discussed in Section  
355 4.6.2. While silicate minerals are common and can provide important natural buffer-  
356 ing, the simulation indicated that, unless the residence time of water traveling through  
357 the treatment zone is greater than approximately one year, the dissolution kinetics for  
358 these minerals are too slow to influence the acidity response. Of the silicate minerals  
359 considered, anorthite dissolution was the fastest, however for 40 mM of dehalogena-  
360 tion occurring over 100 d, the quantity of anorthite predicted to dissolve was two or-  
361 ders of magnitude lower than for calcite (Table 4).

### 362 **4.3 *Influence of electron donor selection***

363 The acidity response and bicarbonate requirements for different electron do-  
364 nors are presented in Fig. 4. The operating conditions for these simulations, with the  
365 exception of the electron donor used, are identical to the base case (Section 3). As  
366 discussed by McCarty et al. (2007), the net acidity generated is directly related to the



367 relative amounts of acetate, carbonate species, and in some cases sodium associated  
368 with the fermentation process (Table 1). The extent of dehalogenation predicted to  
369 occur prior to pH inhibition and the amount of bicarbonate required per mM of deha-  
370 logenation for each electron donor with  $f = 0.2$  (sulfate and iron(III) available) and  $f =$   
371  $0.88$  (sulfate exhausted and  $f$  adjusted based on iron oxide dissolution rate) are shown  
372 in Table 5. Note that lactic acid and glucose generate the same by-products per mol of  
373  $H_2$  (Table 1) and therefore have the same acidity response and bicarbonate require-  
374 ments. Although the pH drop is greatest for these donors (Fig. 4a), the bicarbonate  
375 requirement is largest for butyric acid (Fig. 4b). This is because lactic acid and glu-  
376 cose fermentation adds 0.5 mol of acetate species and 0.5 mol of carbonate species  
377 per mol of  $H_2$ , compared with butyric acid that adds one mol of acetate species and no  
378 carbonate species. The effects of sodium in reducing the acidity generated are evident  
379 in comparing the results for lactic acid to those for sodium lactate. As expected, for all  
380 electron donors the release of  $CO_2$  significantly reduces the bicarbonate requirements  
381 as evident in comparing Fig. 4b and Fig 4c. For all donors, with  $f_{min} = 0.2$ , sulfate is  
382 depleted after 10.9 mM of dehalogenation and as  $f$  switches to 0.88 the additional bi-  
383 carbonate needed per mM of dehalogenation decreases (Table 5, Fig. 4b,c).

384         Of all the electron donors considered, only formate does not require pH buffer-  
385 ing, confirming the observation of McCarty et al. (2007) that it is an excellent choice  
386 in terms of pH control. This is because acetic acid is not produced and the sodium  
387 released is able to neutralize the HCl produced from dehalogenation. For the condi-  
388 tions simulated, the use of formate causes the pH to only decrease to 6.6 for 40 mM of  
389 dehalogenation and therefore no bicarbonate addition is required (Fig. 4). When sul-  
390 fate is present during formate fermentation, the pH increases indicating that the alka-  
391 linity added to the solution from sulfate and iron(III) reduction is greater than the

392 acidity generated from the overall dehalogenation and fermentation reaction. Note that  
393 the results show an initial pH drop (Fig. 4a, dehalogenation < 2.5 mM). This is asso-  
394 ciated with the rapid precipitation of calcite. However the calcium concentration ra-  
395 pidly decreases, as does the rate of calcite precipitation, and this is accompanied by an  
396 increase in pH. Once sulfate is depleted and  $f$  switches to 0.88, the acidity generated  
397 from the overall dehalogenation reaction exceeds the alkalinity added from iron(III)  
398 reduction and the pH gradually decreases (Fig. 4a).

399         This comparison of electron donors assumes that dehalogenation rates and  
400 competition for  $H_2$  are independent of the specific electron donor used. In a detailed  
401 field design, it may be necessary however to account for the characteristics of the  
402 electron donor used. While we have adopted  $f_{min} = 0.2$  for all electron donors, donors  
403 such as glucose, ethanol, methanol, lactic acid and lactate ferment very rapidly and  
404 therefore may have lower  $H_2$  efficiencies of consumption (Fennell et al., 1997; Yang  
405 and McCarty, 2002; AFCEE, 2004). Furthermore, some donors can also ferment via  
406 alternative pathways that result, for example, in the production of propionate (Aulenta  
407 et al., 2007). Both of these effects would likely increase the acidity generated and thus  
408 bicarbonate requirements. A benefit of linoleic acid (vegetable oil emulsions), the  
409 donor used for the base case, is that it only oxidizes under a low  $H_2$  partial pressure  
410 and therefore has a high efficiency of consumption by dehalogenating microorgan-  
411 isms (AFCEE, 2004; Aulenta et al., 2005; Long and Borden, 2006).

#### 412 **4.4 Minimum design pH**

413         While the initial solution pH affects the extent of dehalogenation likely to oc-  
414 cur prior to reaching the inhibitory pH level, the minimum design pH controls the  
415 amount of bicarbonate amendment needed once this level is reached. As shown in Fig.

416 5, the bicarbonate requirement decreases significantly if microorganisms are able to  
417 tolerate more acidic conditions and the design pH can be lowered. With sulfate ex-  
418 hausted and assuming no gas release, the bicarbonate required to maintain pH = 6.2 is  
419 3.1 mM per mM of dehalogenation compared with 7.2 mM per mM of dehalogenation  
420 for pH = 6.7. At low pH, the weak acids added from fermentation and the reduction of  
421 nonchlorinated electron acceptors (e.g., H<sub>2</sub>S) dissociate less and therefore less acidity  
422 (H<sup>+</sup>) is directly added to the solution per mM of dehalogenation. Furthermore, at low-  
423 er pH each mole of bicarbonate added to the system has a greater neutralizing capaci-  
424 ty (2). These points are further discussed in Section 5. The results also indicate that,  
425 due to the elevated concentrations of carbonate species associated with greater bicar-  
426 bonate amendment, gas formation is more likely when more neutral conditions need  
427 to be maintained.

#### 428 **4.5 Use of acetate as electron donor (*p*)**

429 Simulations were performed to determine the bicarbonate requirements when  
430 acetate is used as a direct electron donor for dehalogenation. The parameter *p* is used  
431 to specify the fraction of acetate produced from fermentation of the primary electron  
432 donor is used according to (4). The oxidation of acetate is beneficial because it not  
433 only reduces the total primary electron donor requirement, but as shown in Fig. 6 it  
434 also lowers the overall acidity generated and therefore bicarbonate requirement. In the  
435 absence of gas release, the total bicarbonate required if all of the acetate is used as an  
436 electron donor (*p* = 1) is 3.6 mM per mM of dehalogenation compared with 4.9 mM  
437 per mM of dehalogenation if acetate oxidization is inhibited (*p* = 0). Although two  
438 moles of CO<sub>2</sub> are produced per mol of acetate oxidized, the production of this weak  
439 acid is offset by the consumption of one mole of acetate. With linoelic acid used as

440 the primary electron donor, each mole of acetate oxidized leads to a total reduction of  
441 acetate species of 3.57 mol per mol of dehalogenation. Although the carbonate pro-  
442 duced directly from fermentation and dehalogenation is greater when acetate is oxi-  
443 dized, the simulations indicate that a gas phase forms more rapidly when acetate is not  
444 used. This is because the bicarbonate addition, and therefore the total carbonate spe-  
445 cies added to the system, is much greater for this case. These simulations suggest that  
446 if a conservative estimate is sought with regards to both the primary electron donor  
447 and bicarbonate requirements, it is better to assume that acetate is not used as an elec-  
448 tron donor.

#### 449 **4.6 Influence of nonchlorinated TEAPs**

450 Simulations were performed to investigate the influence of sulfate and  
451 iron(III) reduction on the acidity generated and bicarbonate requirements. Iron(III)  
452 and sulfate reducers are generally the dominant competitors for H<sub>2</sub> with dehalogenat-  
453 ing microorganisms in DNAPL treatment zones. The individual influences of these  
454 TEAPs on the bicarbonate requirements are first examined and then their combined  
455 effects are discussed.

##### 456 **4.6.1 Sulfate reduction**

457 The total bicarbonate requirement as the initial sulfate concentration varies is  
458 shown in Fig. 7a. To examine directly the effects of sulfate reduction on the acidity,  
459 no gas phase is allowed and there are no iron oxides present. For the same  $f_{min}$  (= 0.2),  
460 as the initial sulfate concentration reduces, sulfate is removed from the solution more  
461 rapidly. Once sulfate is removed, as there are no other nonchlorinated electron accep-  
462 tors available,  $f$  switches from 0.2 to 1 and this is accompanied by a decrease in the  
463 bicarbonate required per mM of dehalogenation. This decrease in the bicarbonate re-

464 quired to maintain a constant pH indicates, perhaps surprisingly, that sulfate reduction  
465 may lead to a net addition of acidity. The reduction of one mole of sulfate generates 2  
466 moles of alkalinity, however it also consumes 4 moles of H<sub>2</sub> (Table 2). With linoleic  
467 acid used as the electron donor, to supply 4 moles of H<sub>2</sub>, 2.57 moles of acetic acid are  
468 produced. The generation of this acetic acid, if not oxidized, offsets the alkalinity  
469 benefits of sulfate reduction. The net effect of sulfate reduction, however, will vary  
470 significantly according to the specific electron donor used as each produces different  
471 by-products from fermentation.

472         In these simulations, the initial sulfate availability only influences the extent of  
473 dehalogenation before  $f$  switches to 1. With  $f_{min} = 0.2$ , for initial sulfate concentrations  
474 greater than 40 mM, sulfate is in excess and thus the model predicts the same total  
475 bicarbonate requirement for 40 mM of dehalogenation. Sulfate is likely to be in  
476 excess when sulfate-containing minerals such as gypsum or anhydrite are present, as  
477 these minerals are highly soluble and have rapid dissolution kinetics. This sensitivity  
478 analysis assumes that  $f_{min}$  is independent of sulfate concentration. In reality, the H<sub>2</sub>  
479 directed to sulfate reduction may increase as the sulfate concentration increases, and  
480 thus  $f_{min}$  will decrease. As  $f_{min}$  decreases the bicarbonate required per mM of dehalo-  
481 genation increases due to the net acidity generated from sulfate reduction (Fig. 7b).  
482 However, the amount of H<sub>2</sub> diverted to sulfate reduction and thus the net acidity gen-  
483 erated from this process is constrained by the initial amount of sulfate present. There-  
484 fore, for the same starting sulfate concentration, if all the sulfate is reduced the total  
485 bicarbonate requirement for 40 mM of dehalogenation is identical regardless of  $f_{min}$   
486 (Fig. 7b, c.f.  $f_{min} = 0.2$  and 0.5). As expected, with  $f_{min} = 1$  the bicarbonate requirement  
487 is identical to the case when there is negligible sulfate present.

#### 488 **4.6.2 Iron(III) reduction**

489 The influence of iron(III) reduction on the bicarbonate requirements is illu-  
490 strated in Fig. 8. In these simulations there is negligible sulfate present and gas forma-  
491 tion is not permitted. Although  $f_{min}$  is set at 0.2,  $f$  is adjusted based on the iron oxide  
492 dissolution and thus reduction rate. The results indicate that as the iron oxide reduc-  
493 tion rate increases, the bicarbonate requirement decreases. Each mole of iron oxide  
494 reduced produces two moles of alkalinity, but only 0.5 moles of  $H_2$  are consumed. For  
495 linoleic acid this  $H_2$  demand equates to the production of 0.32 moles of acetic acid.  
496 Thus, in contrast with sulfate reduction, the reduction of iron oxide leads to a net addi-  
497 tion of alkalinity, thus reducing the bicarbonate requirements. For the conditions si-  
498 mulated, the adjusted  $f$  ranges from 1 to 0.78 as  $k$  varies from  $10^{-11}$  to  $10^{-9.75}$  mol m<sup>-2</sup>  
499 s<sup>-1</sup>. The pH is predicted to remain above 6.5 for 40 mM of dehalogenation when  $k$  is  
500 greater than  $10^{-9.5}$  mol m<sup>-2</sup> s<sup>-1</sup>.

501 The iron oxide reduction rate will likely increase as the mass fraction of iron  
502 oxides increases, in particular the fraction of freshly precipitated iron oxides such as  
503 ferrihydrite. Microbial catalysis however also plays an important role in the reduction  
504 of iron oxides with the process significantly enhanced when microbes are in direct  
505 contact with the iron oxide surface (Appelo and Postma, 2005). Although accurately  
506 predicting iron oxide reduction rates is difficult, our results show a consistent trend:  
507 increasing amounts of iron oxide reduction attenuate the amount of bicarbonate  
508 needed for pH control.

#### 509 **4.6.3 Sulfate and iron(III) reduction**

510 Simulations were conducted to examine the influence of the  $H_2$  efficiency with  
511 both sulfate and iron(III) available. The bicarbonate requirements for the base condi-

512 tions with different  $f_{min}$  values adopted are shown in Fig. 9. The combined effects of  
513 sulfate and iron oxide reduction are complex because while sulfate reduction leads to  
514 a net addition of acidity, iron oxide reduction adds alkalinity. Furthermore when both  
515 sulfide and iron(II) are produced, iron sulfides precipitate leading to the addition of  
516 two moles of acidity (5). Based on the model setup, at each reaction step all of the  
517 iron(III) released into the solution (rate controlled, (6)) is reduced in preference to  
518 sulfate and therefore only the  $H_2$  remaining after all the dissolved iron(III) is reduced  
519 is used for sulfate reduction.

520         The alkalinity generated directly from sulfate reduction is consumed if iron  
521 sulfides precipitate. Therefore, the net acidity added from sulfate reduction followed  
522 by iron sulfide precipitation is the 2.57 mol of acetic acid produced in the fermenta-  
523 tion reaction to meet  $H_2$  demand associated with sulfate reduction. As a result, the  
524 bicarbonate requirement is greatest when all the sulfate initially present is reduced and  
525 all of the sulfide produced precipitates (Fig. 9,  $f_{min} = 0.2$ ). As  $f_{min}$  increases, only a  
526 fraction of the sulfate initially present is reduced and subsequently less iron sulfide  
527 precipitates (Fig. 9b,c). This is accompanied by a decrease in the total bicarbonate  
528 required. The bicarbonate requirements are lowest when the surplus  $H_2$  produced from  
529 fermentation directly matches that required to reduce the iron(III) released into the  
530 solution at each reaction step and thus there is no  $H_2$  left over for sulfate reduction  
531 (Fig. 9,  $f_{min} = 0.75$ ). However as  $f_{min}$  approaches unity iron(III) reduction also decreas-  
532 es and as this process adds alkalinity, the bicarbonate requirement increases accor-  
533 dingly.

## 534 5. Simplified Model

535 Based on understanding gained from the PHREEQC model it is possible to  
536 develop a simplified set of equations that may be used for preliminary estimates of the  
537 amount of bicarbonate required once the minimum design pH is reached. At a given  
538 pH, the actual acidity added to the solution per mM of dehalogenation, and thus bi-  
539 carbonate required to match this acidity, depends on the dissociation of the acids add-  
540 ed from fermentation, dehalogenation and the nonchlorinated TEAPs. This dissocia-  
541 tion varies according to pH. The initial solution alkalinity and pH influence the  
542 amount of dehalogenation that will occur prior to the minimum design pH being  
543 reached. Afterwards, however, these parameters do not influence the bicarbonate re-  
544 quirements and so they are not considered in the simplified model. The simulations  
545 also revealed that once bicarbonate addition commences, calcite's influence is not  
546 significant. Therefore, in developing a simplified model for the bicarbonate require-  
547 ment, it is valid to neglect the potential dissolution and/or precipitation of calcite.

548 With linoleic acid used, each mole of dehalogenation adds 0.643 mol of acetic  
549 acid and one mol of HCl (Table 1). For the pH range 6 - 7 it can be assumed that these  
550 acids are completely dissociated and therefore 1.643 mol of  $H^+$  are added per mol of  
551 dehalogenation. To neutralize this acidity it is necessary to add sufficient bicarbonate  
552 such that 1.643 mol of  $CO_2$  will form, thus consuming the  $H^+$  added to the solution  
553 (1). Based on the equilibrium expression describing the dissociation of  $CO_2$  to  $HCO_3^-$   
554 (2), the total bicarbonate needed to neutralize one mol of acidity is  $1 + 10^{pH-6.3}$ . There-  
555 fore with linoleic acid used as the electron donor, the bicarbonate required to maintain  
556 a constant pH for one mol of dehalogenation ( $R_{dehalogenation}$ ) is:

$$557 R_{dehalogenation} = 1.643(1 + 10^{pH-6.3}). \quad (7)$$



558 The net acidity added to the solution and thus bicarbonate requirement associated with  
559 sulfate reduction can also be calculated. The dissociation constant for H<sub>2</sub>S is 10<sup>-7.02</sup>  
560 and so the reduction of one mol of sulfate produces  $\frac{1}{10^{7.02-\text{pH}} + 1}$  mol of HS<sup>-</sup> and  
561  $1 - \frac{1}{10^{7.02-\text{pH}} + 1}$  mol of H<sub>2</sub>S. S<sup>2-</sup> is negligible at pH between 6 - 7. In consequence, sul-  
562 fate reduction directly adds  $2 - \frac{1}{10^{7.02-\text{pH}} + 1}$  mol of alkalinity. However 4 mol of H<sub>2</sub>  
563 are consumed for each mol of sulfate reduced and with linoleic acid used as the elec-  
564 tron donor, this is associated with the production of 2.57 mol of acetic acid and thus  
565 the addition of 2.57 mol of acidity. Thus, the bicarbonate required per mol of sulfate  
566 reduced ( $R_{\text{sulfate}}$ ) is given by:

$$567 \quad R_{\text{sulfate}} = \left( 0.57 + \frac{1}{10^{7.02-\text{pH}} + 1} \right) (1 + 10^{\text{pH}-6.3}). \quad (8)$$

568 In a similar manner it can be determined that with linoleic acid, the bicarbonate re-  
569 quired per mol of iron oxide reduced ( $R_{\text{iron}}$ ) is:

$$570 \quad R_{\text{iron}} = -1.679 (1 + 10^{\text{pH}-6.3}). \quad (9)$$

571 This equation illustrates, as previously shown in Section 4.6.2, that iron(III) reduction  
572 may decrease the bicarbonate requirement. Finally, iron sulfide precipitation removes  
573 the H<sub>2</sub>S and HS<sup>-</sup> produced from sulfate reduction and thus the acidity added when this  
574 precipitate forms is identical to the alkalinity added directly from sulfate reduction.  
575 Therefore, the bicarbonate required per mol of iron sulfide that precipitates ( $R_{\text{FeS}}$ ) is:

$$576 \quad R_{\text{FeS}} = \left( 2 - \frac{1}{10^{7.02-\text{pH}} + 1} \right) (1 + 10^{\text{pH}-6.3}). \quad (10)$$

577 For a given amount of sulfate and iron(III) reduction, and iron sulfide precipitation per  
578 mol of dehalogenation, (7) to (10) can be used to estimate the overall bicarbonate re-  
579 quirement ( $= R_{dehalogenation} + R_{sulfate} + R_{iron} + R_{FeS}$ ). As (7) to (10) are based on the dis-  
580 sociation of acids at a constant pH, they are only applicable once the minimum design  
581 pH is reached. They do not allow prediction of the extent of dehalogenation likely to  
582 occur prior to this pH being reached. Potential gas bubble formation and use of acetate  
583 as an electron donor ( $p = 0$ ) are neglected also, the implications of which are dis-  
584 cussed below.

585 We now use this simplified model to estimate the bicarbonate requirements for  
586 the base conditions and compare the results to the PHREEQC geochemical model  
587 predictions (Section 4.1). The amount of iron(III) and sulfate reduction, and iron sul-  
588 fide precipitating per mol of dehalogenation must first be estimated. Assuming the  
589 surface area of iron oxide is constant, from (6) the rate of iron oxide reduction at a pH  
590  $= 6.5$  with  $k = 10^{-10.2}$  can be approximated as  $0.106 \text{ mM d}^{-1}$ . For the base conditions  
591 the dehalogenation rate is  $0.4 \text{ mM d}^{-1}$  (40 mM of dehalogenation occurs over 100 d).  
592 By comparing these time scales it can be determined that 0.265 mM of iron(III) will  
593 be reduced per mM of dehalogenation. With the same  $f_{min}$  ( $= 0.2$ ), 4 mM  $\text{H}_2$  per mM  
594 of dehalogenation are available for the reduction of nonchlorinated electron acceptors.  
595 With 0.265 mM of iron(III) reduced, 3.87 mM of  $\text{H}_2$  are available for sulfate reduc-  
596 tion and this equates to the reduction of 0.976 mM of sulfate per mM of dehalogena-  
597 tion. The precipitation of iron sulfides is limited by the iron(II) availability and there-  
598 fore 0.265 mM of iron sulfide will form per mM of dehalogenation. Thus, for the base  
599 conditions with sulfate available, using (7) to (10) and with a pH = 6.5, the bicarbo-  
600 nate required per mM of dehalogenation is estimated at 6.3 mM. When the sulfate is  
601 removed the amount of iron(III) reduced per mM of dehalogenation will remain at

602 0.265 mM. For 40 mM of dehalogenation and this rate of iron(III) reduction, there  
603 will be sufficient sulfide available in solution, even once sulfate is exhausted, for iron  
604 sulfide to continue to precipitate as iron(II) is produced (i.e., 0.265 mM of iron sulfide  
605 precipitate per mM of dehalogenation). In applying (7), (9) and (10) the bicarbonate  
606 required is estimated at 4.3 mM per mM of dehalogenation. In comparing these esti-  
607 mates with the PHREEQC model predictions (Table 5), it can be seen that the simpli-  
608 fied model under predicts the bicarbonate requirements. As potential gas release and  
609 acetate oxidization are also neglected in the PHREEQC simulations used for this  
610 comparison, the underestimation is primarily due to the extensive speciation processes  
611 included in the PHREEQC model (e.g., formation of aqueous species such as NaH-  
612 CO<sub>3</sub> and KHCO<sub>3</sub>).

613 Equations (7) - (10) assume that linoleic acid is used as the electron donor,  
614 however similar equations have been developed (Table 6) for all common electron  
615 donors listed in Table 1. For lactic acid, glucose and methanol the CO<sub>2</sub> produced from  
616 fermentation is included in the simplified model as this increases the bicarbonate re-  
617 quirement. In a similar manner, the HCO<sub>3</sub><sup>-</sup> produced upon fermentation of sodium  
618 lactate is also considered. A comparison of the bicarbonate requirements predicted  
619 using the geochemical model and using these equations for each electron donor is  
620 shown in Table 5 for the two different  $f$  values. For all donors considered, the compar-  
621 ison is reasonable with the simplified approach generally under predicting the bicar-  
622 bonate requirement relative to the PHREEQC model, typically by between 15 and  
623 20%. The difference in the estimates increases as the bicarbonate requirement de-  
624 creases (i.e., methanol for  $f_{min} = 0.2$ ). This is because the aqueous speciation processes  
625 included in PHREEQC have a greater relative impact as the bicarbonate requirement  
626 decreases. The simplified model does not include the use of acetate as an electron

627 donor or the release of CO<sub>2</sub>. As these processes both reduce the bicarbonate require-  
628 ments (e.g., see Fig. 3b and Fig. 6, respectively) and will likely occur, the lower sim-  
629 plified model estimates may actually be more in line with field conditions. Therefore,  
630 for a preliminary design estimate, the expressions in Table 6 provide a simple means  
631 to estimate the field bicarbonate requirements. The simplified model also provides  
632 important quantitative understanding of the processes influencing the amount of acidi-  
633 ty generated (e.g., electron donor selection). For more detailed design however, the  
634 more detailed modeling approach might be considered.

## 635 **6. Conclusions**

636 This study provides insight into the acidity generated and the bicarbonate addi-  
637 tion required to maintain the pH in the DNAPL source zone within the optimal range  
638 for dehalogenating bacteria. The major findings are outlined below.

- 639 • Where extensive dehalogenation is likely to occur in the DNAPL source zone,  
640 significant bicarbonate addition may be necessary even in soils that are natu-  
641 rally well-buffered. While calcite provides some pH control, its buffering ca-  
642 pacity is limited by solubility constraints and may not be sufficient to prevent  
643 acidic conditions developing.
- 644 • The choice of electron donor strongly influences the bicarbonate requirements  
645 due to the relative amounts of acetate, carbonate species and sodium asso-  
646 ciated with the fermentation process (Table 1).
- 647 • The bicarbonate required per mM of dehalogenation depends not only on the  
648 electron donor fermentation and dehalogenation processes but also on the  
649 competing nonchlorinated TEAPs. Although sulfate and iron oxide reduction  
650 both add alkalinity to the solution (Table 2), these alkalinity benefits can be

651 counterbalanced by the acidity (e.g., acetic acid) added in producing the H<sub>2</sub>  
652 consumed by these TEAPs. Whether sulfate and iron(III) reduction lead to a  
653 net generation of alkalinity depends on the specific electron donor used (Table  
654 6). If both iron(III) and sulfate reduction occur, iron sulfides are likely to pre-  
655 cipitate and this adds acidity to the solution, thus increasing the bicarbonate  
656 requirement.

- 657 • The formation of a gas phase and thus the release of CO<sub>2</sub> lowers the bicarbo-  
658 nate required per mM of dehalogenation due to the shift in the dissolved car-  
659 bonate equilibria (2).
- 660 • The bicarbonate requirement depends strongly on the minimum design pH  
661 with the requirement increasing significantly with increase in design pH to-  
662 wards the more neutral value more favored by dehalogenating bacteria.

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807

808 **Table 1.** Fermentation reactions for common electron donors and amounts of carbonate species ( $\Sigma[\text{CO}_2 + \text{HCO}_3^-]$ ), acetate species ( $\Sigma$   
809  $[\text{CH}_3\text{COOH} + \text{CH}_3\text{COO}^-]$ ) and sodium ( $\text{Na}^+$ ) added per mol of  $\text{H}_2$  produced. The total moles of by-products added per mol of dehalo-  
810 genation can be calculated by multiplying the amounts provided by  $1/f$ .

Electron donor	Fermentation reaction	Overall reaction for dehalogenation and fermentation	$\Sigma(\text{CO}_2 + \text{HCO}_3^-)$	$\Sigma(\text{CH}_3\text{COOH} + \text{CH}_3\text{COO}^-)$	$\text{Na}^+$
Linoleic acid	$\text{C}_{18}\text{H}_{32}\text{O}_2 + 16\text{H}_2\text{O} = 14\text{H}_2 + 9\text{CH}_3\text{COOH}$	$\text{R-Cl} + \frac{1}{14f} \text{C}_{18}\text{H}_{32}\text{O}_2 + \frac{8}{7f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{9}{14f} \text{CH}_3\text{COOH} + \frac{(1-f)}{f} \text{H}_2$	0	0.643	0
Sodium Lactate	$\text{CH}_3\text{CHOHCOONa} + 2\text{H}_2\text{O} = 2\text{H}_2 + \text{CH}_3\text{COOH} + \text{NaHCO}_3$	$\text{R-Cl} + \frac{1}{2f} \text{CH}_3\text{CHOHCOONa} + \frac{1}{f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{1}{2f} \text{CH}_3\text{COOH} + \frac{1}{2f} \text{NaHCO}_3 + \frac{(1-f)}{f} \text{H}_2$	0.5	0.5	0.5
Lactic acid	$\text{CH}_3\text{CHOHCOOH} + \text{H}_2\text{O} = 2\text{H}_2 + \text{CH}_3\text{COOH} + \text{CO}_2$	$\text{R-Cl} + \frac{1}{2f} \text{CH}_3\text{CHOHCOOH} + \frac{1}{2f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{1}{2f} \text{CH}_3\text{COOH} + \frac{1}{2f} \text{CO}_2 + \frac{(1-f)}{f} \text{H}_2$	0.5	0.5	0
Glucose	$\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} = 4\text{H}_2 + 2\text{CH}_3\text{COOH} + 2\text{CO}_2$	$\text{R-Cl} + \frac{1}{4f} \text{C}_6\text{H}_{12}\text{O}_6 + \frac{1}{2f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{1}{2f} \text{CH}_3\text{COOH} + \frac{1}{2f} \text{CO}_2 + \frac{(1-f)}{f} \text{H}_2$	0.5	0.5	0
Butyric acid	$\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} = 2\text{H}_2 + 2\text{CH}_3\text{COOH}$	$\text{R-Cl} + \frac{1}{2f} \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + \frac{1}{f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{1}{f} \text{CH}_3\text{COOH} + \frac{(1-f)}{f} \text{H}_2$	0	1	0

---

Methanol	$\text{CH}_3\text{OH} + \text{H}_2\text{O} = 3\text{H}_2 + \text{CO}_2$	$\text{R-Cl} + \frac{1}{f} \text{CH}_3\text{OH} + \frac{1}{3f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{1}{3f} \text{CO}_2 + \frac{(1-f)}{f} \text{H}_2$	0.33	0	0
Ethanol	$\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} = 2\text{H}_2 + \text{CH}_3\text{COOH}$	$\text{R-Cl} + \frac{1}{2f} \text{CH}_3\text{CH}_2\text{OH} + \frac{1}{2f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{1}{2f} \text{CH}_3\text{COOH} + \frac{(1-f)}{f} \text{H}_2$	0	0.5	0
Formate	$\text{HCOONa} + \text{H}_2\text{O} = \text{NaHCO}_3 + \text{H}_2$	$\text{R-Cl} + \frac{3}{14f} \text{HCOONa} + \frac{1}{f} \text{H}_2\text{O} = \text{R-H} + \text{HCl} + \frac{1}{f} \text{NaHCO}_3 + \frac{(1-f)}{f} \text{H}_2$	1	0	1

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811 **Table 2.** Terminal electron-accepting processes (TEAPs) and amounts of alkalinity (OH<sup>-</sup>)  
 812 ) produced and H<sub>2</sub> consumed per mol of electron acceptor reduced.

	Reaction	Alkalinity added (per mol)	H <sub>2</sub> consumed (per mol)
Oxygen reduction	$O_2 + 2H_2 = 2H_2O$	0	2
Nitrate reduction	$2NO_3^- + 5H_2 = N_2 + 2OH^- + 4H_2O$	1	2.5
Iron oxide reduction			
Goethite	$2FeOOH(s) + H_2 = 2Fe^{2+} + 4OH^-$	2	0.5
Ferrihydrite	$2Fe(OH)_3(s) + H_2 = 2Fe^{2+} + 4OH^- + 2H_2O$	2	0.5
Dehalogenation	$R-Cl + H_2 = R-H + H^+ + Cl^-$	-1	1
Sulfate reduction	$SO_4^{2-} + 4H_2 = H_2S + 2OH^- + 2H_2O$	2	4
Methanogenesis	$CO_2 + 4H_2 = CH_4 + 2H_2O$	0	4

813

814 **Table 3.** Initial groundwater composition for base case.

Constituent	Concentration
pH	7
Alkalinity	220 mg L <sup>-1</sup> CaCO <sub>3</sub>
Ca <sup>2+</sup>	8.1 mM
Cl <sup>-</sup>	9.0 mM
K <sup>+</sup>	2.0 mM
Mg <sup>2+</sup>	5.0 mM
Na <sup>+</sup>	6.0 mM
SO <sub>4</sub> <sup>2-</sup>	10.4 mM
N <sub>2</sub>	Partial pressure = 0.79

815

816 **Table 4.** Amounts of mineral dissolved for 40 mM of dehalogenation. The initial moles  
817 of mineral present per kg of water are typical and are based on the mineralogy at a con-  
818 taminated chlorinated solvent site currently undergoing enhanced bioremediation as part  
819 of the SABRE project.

Mineral	Initial amount present (mol kg of water <sup>-1</sup> )	Amount dissolved (mol kg of water <sup>-1</sup> )
Calcite	0.5	$3.87 \times 10^{-2}$
Gypsum	0.1	$1.83 \times 10^{-2}$
Goethite	3.3	$1.71 \times 10^{-2}$
Anorthite	0.5	$4.02 \times 10^{-4}$
Albite	0.16	$1.55 \times 10^{-5}$
K-Feldspar	0.43	$1.33 \times 10^{-5}$
Illite	0.41	$1.27 \times 10^{-5}$
Chlorite	0.04	$1.26 \times 10^{-5}$

820  
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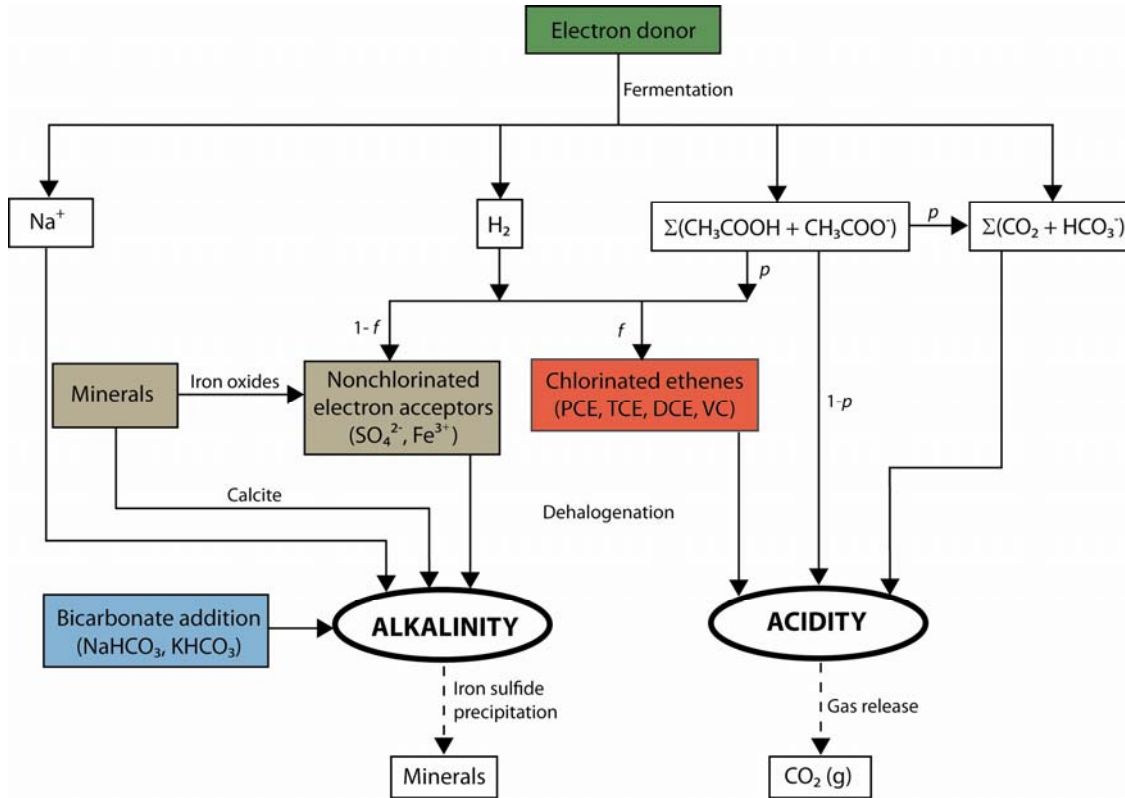
822 **Table 5.** Predicted extent of dehalogenation that occurs while lowering the pH from the  
823 initial value of 7 to the design value of 6.5, and the bicarbonate required per mM of de-  
824 halogenation to maintain pH = 6.5 as calculated using the PHREEQC model, and the  
825 simplified model (Section 5). Results are for the base case discussed in Section 4.1.

	PHREEQC model			Simplified model (Section 5)	
	Dehalogenation before pH < 6.5 (mM)	Bicarbonate required for $f =$ 0.2 (mM)	Bicarbonate required for $f =$ 0.88 (mM)	Bicarbonate required for $f =$ 0.2 (mM)	Bicarbonate required for $f =$ 0.88 (mM)
Linoleic acid	4.5	7.5	4.9	6.3	4.3
Sodium lactate	5	3.3	4.0	2.0	3.3
Lactic ac- id/Glucose	2.2	10	5.8	8.8	5.1
Butyric acid	2.7	12.8	6.4	11.0	5.4
Methanol	7.6	2.6	3.5	0.6	3.0
Ethanol	6.5	5.8	4.5	4.5	3.9
Formate	pH = 6.6 after 40 mM of dehalogenation				

826 **Table 6.** Simplified model to calculate the net acidity added per mol of dehalogenation, sulfate reduction, iron oxide reduction and  
 827 iron sulfide precipitation for common electron donors at the minimum design pH. The acidity generated is multiplied by  $1 + 10^{\text{pH}-6.3}$  to  
 828 calculate the total bicarbonate required per mol of dehalogenation. These equations neglect potential gas release and acetate oxidiza-  
 829 tion.

	Dehalogenation	Sulfate reduction	Iron oxide reduction	Iron sulfide precipitation
Linoleic acid	1.643	$0.57 + \frac{1}{10^{7.02-\text{pH}} + 1}$	-1.679	$2 - \frac{1}{10^{7.02-\text{pH}} + 1}$
Lactic acid/Glucose	$1.5 + 0.5 \frac{10^{\text{pH}-6.3}}{10^{\text{pH}-6.3} + 1}$	$2 \frac{10^{\text{pH}-6.3}}{10^{\text{pH}-6.3} + 1} + \frac{1}{10^{7.02-\text{pH}} + 1}$	$0.25 \frac{10^{\text{pH}-6.3}}{10^{\text{pH}-6.3} + 1} - 1.75$	$2 - \frac{1}{10^{7.02-\text{pH}} + 1}$
Sodium lactate	$1.5 - \frac{0.5}{10^{\text{pH}-6.3} + 1}$	$-\frac{2}{10^{\text{pH}-6.3} + 1} + \frac{1}{10^{7.02-\text{pH}} + 1}$	$-\frac{0.25}{10^{\text{pH}-6.3} + 1} - 1.75$	$2 - \frac{1}{10^{7.02-\text{pH}} + 1}$
Butyric acid	2	$2 + \frac{1}{10^{7.02-\text{pH}} + 1}$	-1.5	$2 - \frac{1}{10^{7.02-\text{pH}} + 1}$
Methanol	$1 + 0.33 \frac{10^{\text{pH}-6.3}}{10^{\text{pH}-6.3} + 1}$	$1.33 \frac{10^{\text{pH}-6.3}}{10^{\text{pH}-6.3} + 1} + \frac{1}{10^{7.02-\text{pH}} + 1} - 2$	$0.167 \frac{10^{\text{pH}-6.3}}{10^{\text{pH}-6.3} + 1} - 2$	$2 - \frac{1}{10^{7.02-\text{pH}} + 1}$
Ethanol	1.5	$\frac{1}{10^{7.02-\text{pH}} + 1}$	-1.75	$2 - \frac{1}{10^{7.02-\text{pH}} + 1}$

830



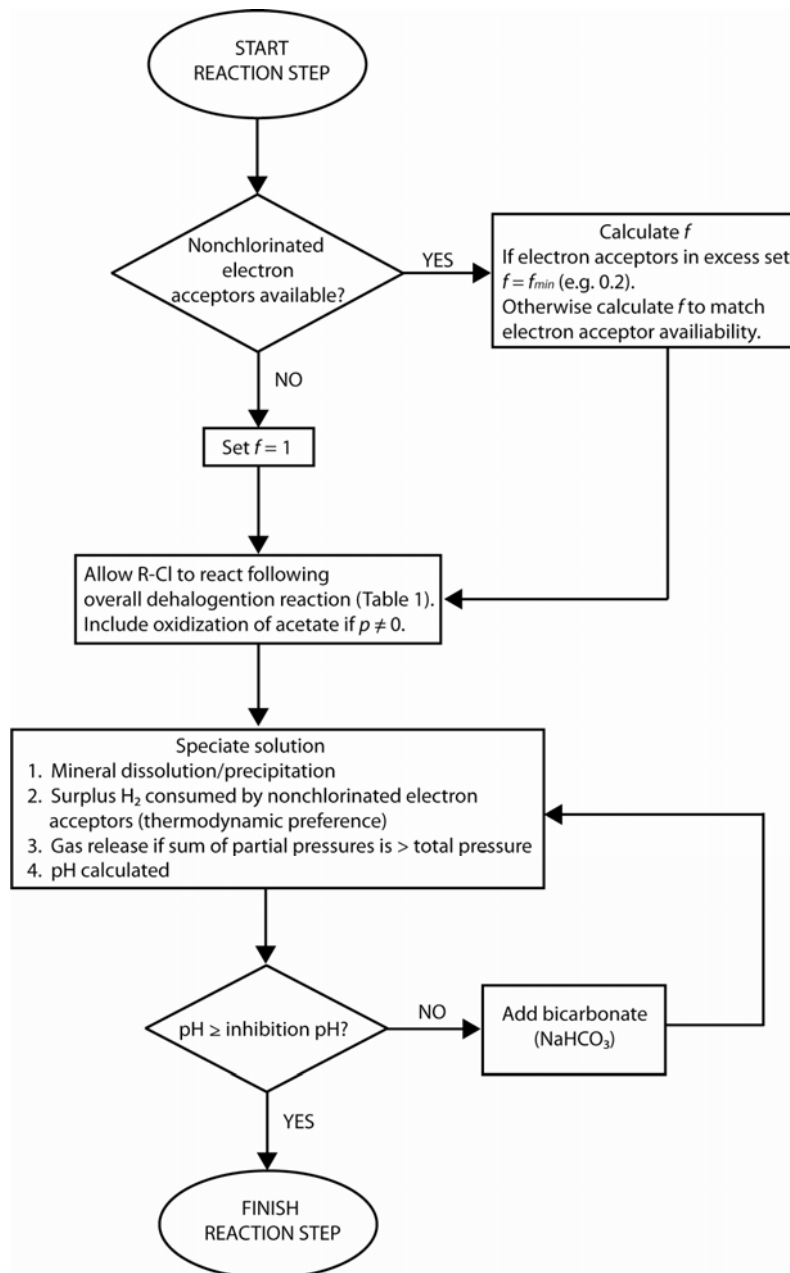
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832 **Figure 1.** Main factors influencing the solution acidity and alkalinity. To maintain a con-

833 stant pH the acidity and alkalinity additions (including bicarbonate addition) must bal-

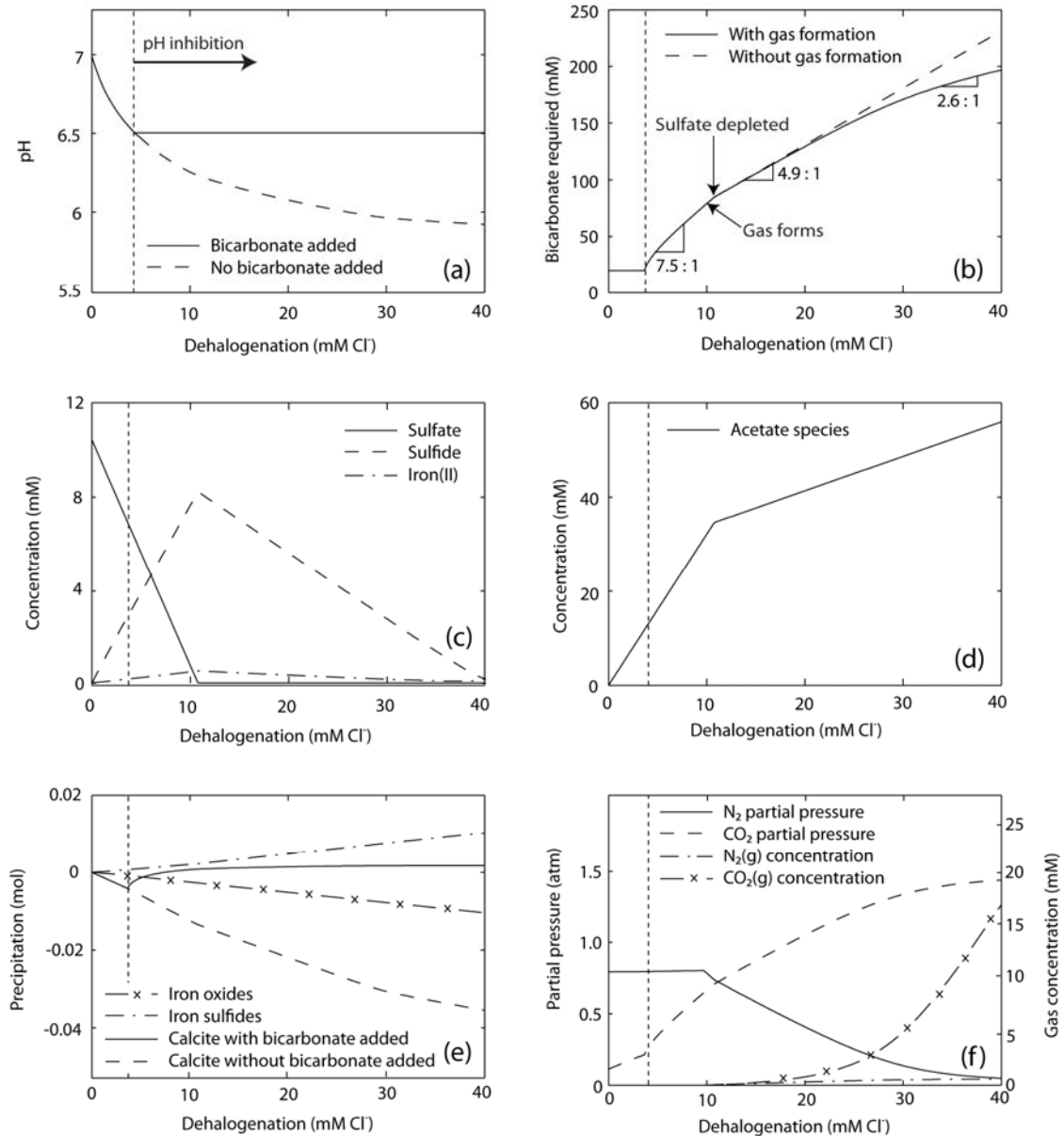
834 ance. A description of the processes is provided in Section 2.

835



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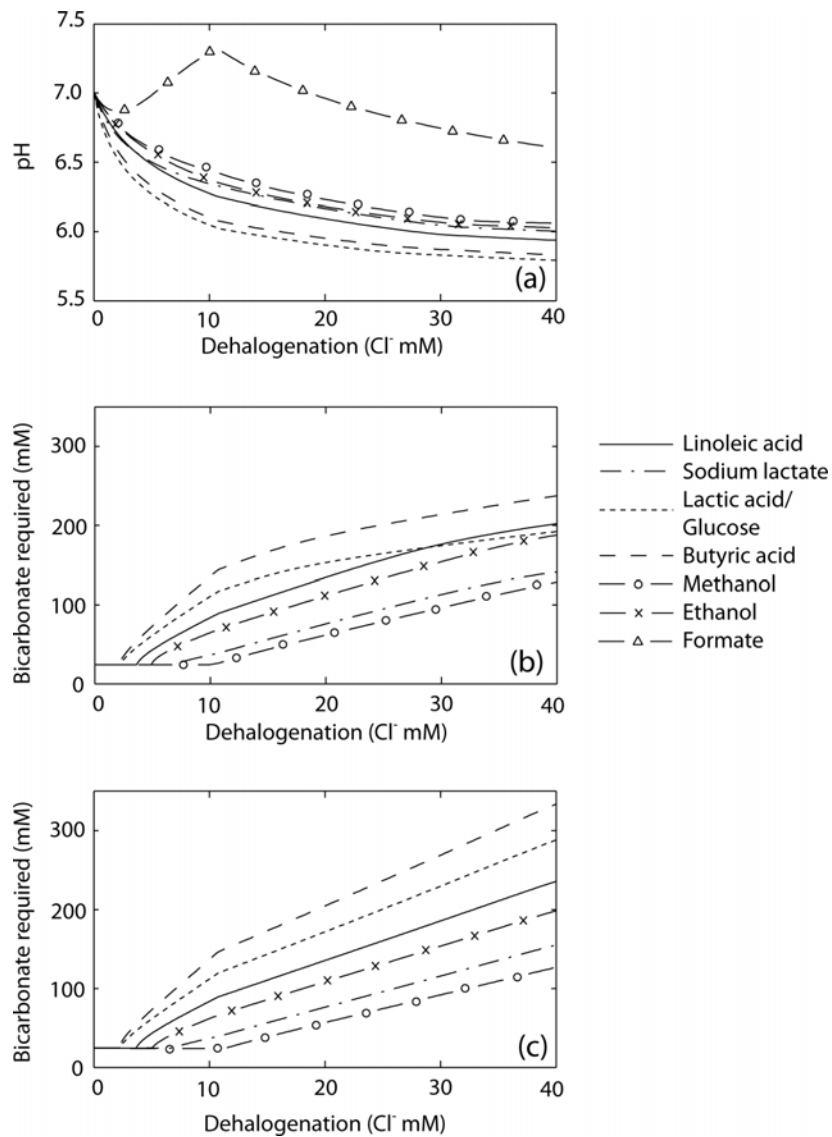
837 **Figure 2.** Flow chart of model algorithm for each reaction step. For the simulations pre-  
 838 sented, the dehalogenation of 40 mM of chlorinated ethene equivalents occurs over 500  
 839 reaction steps and therefore at each step 40/500 mM of R-Cl is allowed to react according  
 840 to the overall dehalogenation and fermentation reaction (Table 1). A description of the  
 841 algorithm is provided in Section 2.



842

843 **Figure 3.** Effect of the extent of dehalogenation for the base conditions with linoleic acid  
 844 used as the electron donor on (a) pH with and without bicarbonate addition, (b) bicarbo-  
 845 nate required to maintain pH at or above 6.5, (c) concentrations of sulfate, sulfide and  
 846 iron(II) with bicarbonate addition, (d) concentration of acetate species with bicarbonate  
 847 addition, (e) change in calcite without bicarbonate addition and change in calcite, iron  
 848 oxides and iron sulfides with bicarbonate addition, and (f) partial pressures of N<sub>2</sub> and

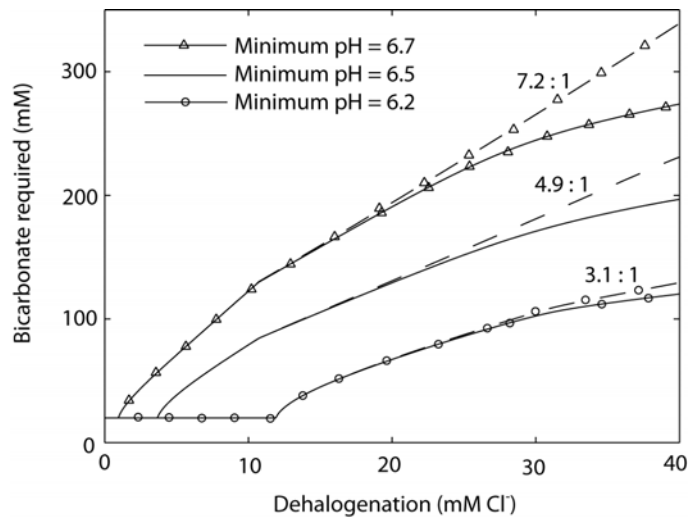
849  $\text{CO}_2$  and concentrations of  $\text{N}_2(\text{g})$  and  $\text{CO}_2(\text{g})$  with bicarbonate addition. The ratios listed  
850 below the curve in (b) give the amount of bicarbonate (mM) required per mM of dehalo-  
851 genation (Section 4.1). Note that in (e) negative precipitation implies dissolution. Vertical  
852 dashes in each plot indicate the point at which pH control is necessary.  
853



854

855 **Figure 4.** Effect of different electron donors on the (a) pH without bicarbonate addition,  
 856 (b) bicarbonate required to maintain pH at or above 6.5 with gas release, and (c) bicarbo-  
 857 nate required to maintain pH at or above 6.5 without gas release. Note that the amount of  
 858 bicarbonate required when formate is used is zero and therefore formate is not plotted in  
 859 (b) and (c).

860

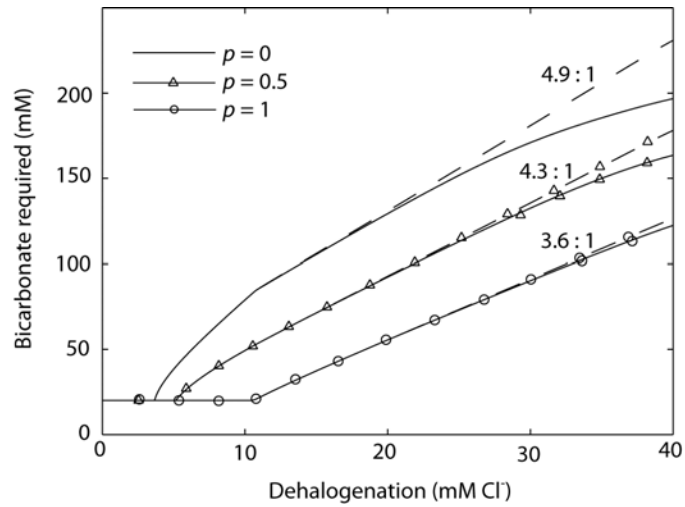


861

862 **Figure 5.** Bicarbonate required as dehalogenation proceeds for different minimum design  
 863 pH values. Results are for the base conditions in which linoleic acid is the electron donor.  
 864 For each minimum pH the bicarbonate required when no gas is allowed to form are also  
 865 shown (dashed lines). The amounts of bicarbonate required per mM of dehalogenation  
 866 when there is no gas release and  $f = 0.88$  are listed above the curves.

867

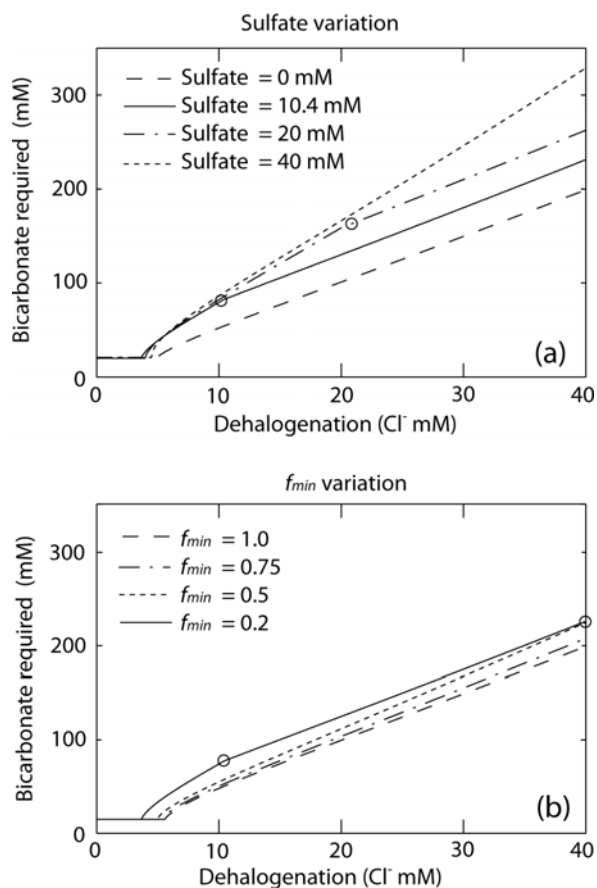




868

869 **Figure 6.** Bicarbonate required to maintain the pH at or above 6.5 with  $p = 0$  (no acetate  
 870 oxidation),  $p = 0.5$  and  $p = 1$  (complete oxidation of acetate) as dehalogenation proceeds.  
 871 Results are for the base conditions in which linoleic acid is the electron donor. For each  
 872 value of  $p$ , the amount of bicarbonate required when no gas is allowed to form is also  
 873 shown (dashed lines). The amounts of bicarbonate required per mM of dehalogenation  
 874 when there is no gas release and  $f = 0.88$  are listed above the curves.

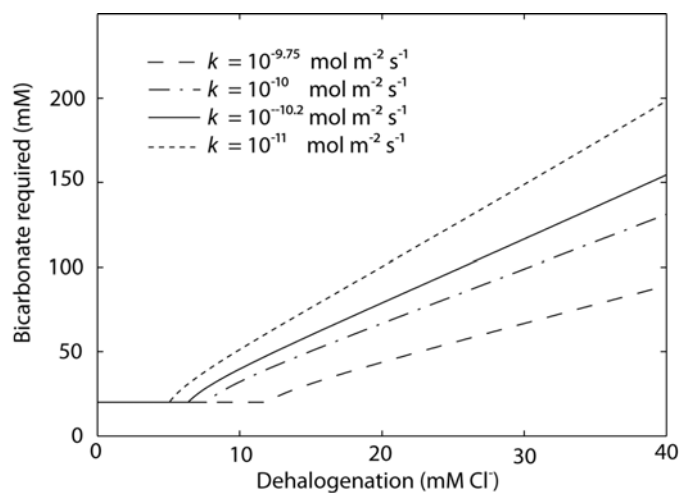
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876

877 **Figure 7.** Influence of (a) initial sulfate concentration and (b) minimum  $H_2$  efficiency  
 878 ( $f_{min}$ ) on the bicarbonate required to maintain the pH at or above 6.5 with sulfate reduc-  
 879 tion the sole nonchlorinated TEAP. Other than the variation of these parameters and ab-  
 880 sence of iron oxides and gas formation, the results are for the base conditions in which  
 881 linoleic acid is the electron donor and sulfate is present initially at 10.4 mM. The open  
 882 circles (○) show the points where sulfate is exhausted and  $f$  switches to 1.

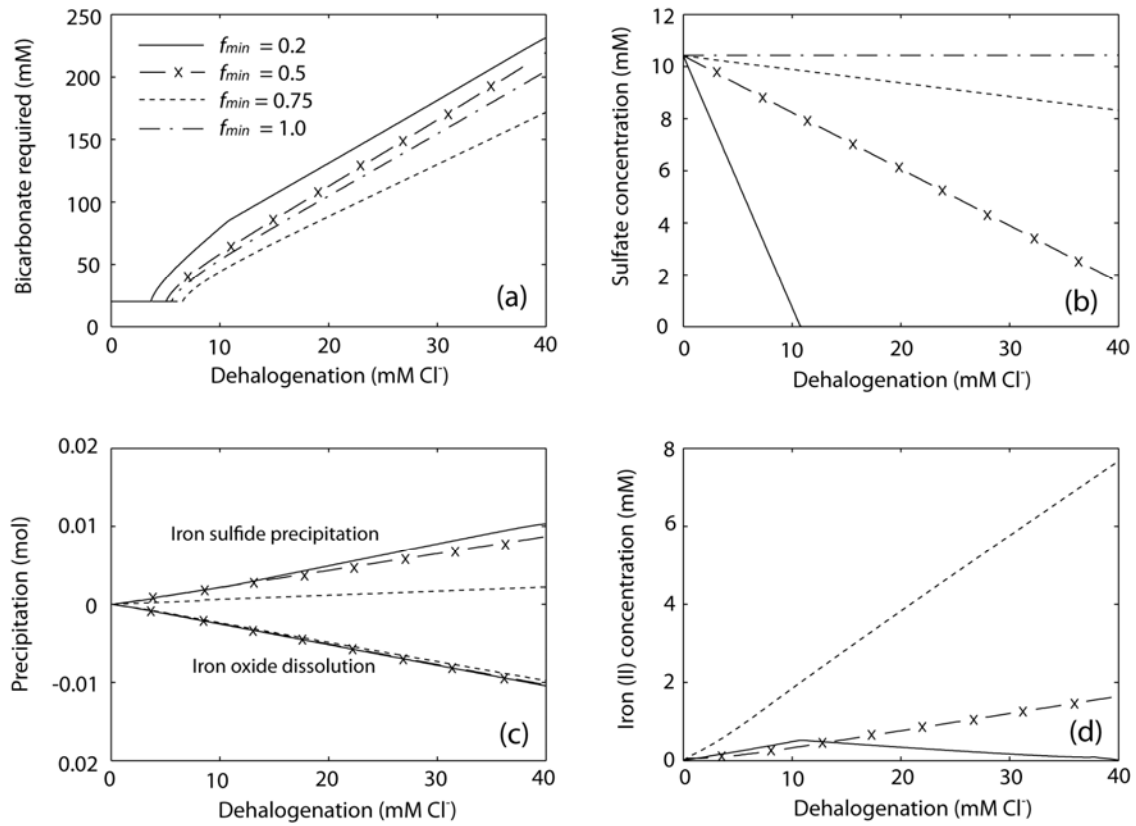
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884

885 **Figure 8.** Influence of iron oxide dissolution and reduction rate constant ( $k$ ) on bicarbo-  
 886 nate required to maintain the pH at or above 6.5 with iron(III) reduction the sole nonchlo-  
 887 rinated TEAP. Other than the variation of  $k$  and absence of sulfate and gas formation, the  
 888 results are for the base conditions in which linoleic acid is the electron donor.

889



890

891 **Figure 9.** Influence of minimum H<sub>2</sub> efficiency ( $f_{min}$ ) on (a) bicarbonate required to main-  
 892 tain the pH at or above 6.5, (b) sulfate concentration, (c) iron oxide dissolution and iron  
 893 sulfide precipitation, and (d) iron(II) concentration. Results are shown for the base condi-  
 894 tions in which linoleic acid is the electron donor. For  $f_{min} = 1.0$ , there is negligible iron  
 895 oxide dissolution and therefore negligible iron sulfide precipitation and concentration of  
 896 iron(II).