Available online at www.sciencedirect.com
ScienceDirect
European Journal of Soil Biology 43 (2007) 276–282
EUROPEAN JOURNAL OF SOIL BIOLOGY
http://www.elsevier.com/locate/ejsobi

Original article

Electron donor and pH relationships for biologically enhanced dissolution of chlorinated solvent DNAPL in groundwater

Perry L. McCarty*, Min-Ying Chu, Peter K. Kitanidis

Department of Civil and Environmental Engineering, Stanford University, Stanford, CA 94305, USA

Available online 26 March 2007

Abstract

Biologically enhanced dissolution offers a method to speed removal of chlorinated solvent dense non-aqueous-phase liquid (DNAPL) sources such as tetrachloroethene (PCE) and trichoroethene (TCE) from aquifers. Bioremediation is accomplished by adding an electron donor to the source zone where fermentation to intermediates leading to acetic acid and hydrogen results. The hydrogen and possibly acetic acid are used by dehalogenating bacteria to convert PCE and TCE to ethene and hydrochloric acid. Reductive dehalogenation is thus an acid forming process, and sufficient alkalinity must be present to maintain a near neutral pH. The bicarbonate alkalinity required to maintain pH above 6.5 is a function of the electron donor: 800 mg/L of bicarbonate alkalinity is sufficient to achieve about 1.2 mM TCE dechlorination with glucose, 1.7 mM with lactate, and a much higher 3.3 mM with formate. Laboratory studies indicate that in mixed culture, formate can be used as an electron donor for complete conversion to ethene, contrary to pure cultures studies indicating it cannot. Various strategies can be used to add electron donor to an aquifer for DNAPL dehalogenation while minimizing pH problems and excessive electron donor usage, including use of injection-extraction wells, dual recirculation wells, and nested injection-extraction wells.

Keywords: DNAPL; Dehalogenation; Tetrachloroethene; Trichloroethene; pH; Alkalinity; Bioremediation; Electron donor; Formate; Glucose; Lactate; Hydrogen; Groundwater

1. Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) are the most frequently found and costly to control organic contaminants in groundwater. Chlorinated solvent spills migrate downward to form dense non-aqueous phase liquids (DNAPLs), which constitute sources of contamination to groundwater that may last for decades, if not centuries. Recent research has indicated that near-saturation concentrations of chlorinated solvents can be biodegraded by specialized anaerobic microorganisms that use the chlorinated solvents as electron acceptors in energy metabolism [13,14]. These organisms require an electron donor, which may be present in the aquifer, leading to natural attenuation, or most often, must be added as part of an engineered bioremediation scheme. Efforts based upon this research are now being directed towards use of biodegradation to reduce the life span of chlorinated solvent DNAPLs.

Among the advantages of chlorinated solvent DNAPL biodegradation [13] are: (1) it can result in

* Corresponding author. Fax: +1 650 725 3164.
E-mail address: pmccarty@stanford.edu (P.L. McCarty).

1164-5563/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejsobi.2007.03.004

enhanced rates of solvent dissolution, especially for PCE; (2) the high chlorinated solvent concentrations near the DNAPL and their degradation products as well are toxic to microorganisms, such as methanogens, that otherwise compete with dechlorinating microorganisms for the electron donor; and (3) the costs for delivery of the electron donor per unit of solvent degraded are much less when applied to high solvent concentrations. The question then arises as to what are the best donors to use for chlorinated solvent dehalogenation and what are the best strategies for their delivery to the DNAPL source area?

2. Electron donors

Reductive dehalogenation of PCE occurs in a stepwise fashion, generally with hydrogen as the preferred electron donor although acetic and other organic acids may be used by some dehalogenating microorganisms [6,11,12], converting PCE to TCE to 1,2-*cis*-dichloroethene (cDCE) to vinyl chloride (VC), and finally to ethene.

Reactions when using hydrogen for reductive Dehalogenation of PCE are:

$$CCl_2{=}CCl_2 + H_2 = CHCl{=}CCl_2 + HCl \quad (1)$$

$$CHCl{=}CCl_2 + H_2 = CHCl{=}CHCl + HCl \quad (2)$$

$$CHCl{=}CHCl + H_2 = CH_2{=}CHCl + HCl \quad (3)$$

$$CH_2{=}CHCl + H_2 = CH_2{=}CH_2 + HCl \quad (4)$$

$$\text{Net:}\quad CCl_2{=}CCl_2 + 4H_2 = CH_2{=}CH_2 + 4HCl \quad (5)$$

Different microorganisms have different abilities to use PCE, TCE, cDCE, and VC in energy metabolism. There are several different bacterial genera capable of using PCE and TCE, but only *Dehalococcoides* has been found to use cDCE and VC in energy metabolism [3–5,9]. Interestingly, different *Dehalococcoides* strains are restricted in the chlorinated species that they can dehalogenate. Thus, in order to obtain complete dehalogenation efficiently, a strain that uses VC in energy metabolism is required [3,5].

Hydrogen itself may be injected into an aquifer, but has several disadvantages. It has low solubility (about 1 mM), which can be a significant limitation for DNAPL dehalogenation. It is a hazardous compound, and as indicated in Eqs. (1)–(5), an end product of dehalogenation is hydrochloric acid. The high hydrochloric acid production can be a significant problem, necessitating a high buffer or neutralization capacity to prevent adverse pH conditions [1].

Most frequently, organic compounds are used for electron donors. Here, fermentation leads to the production of acetate and hydrogen, as indicated in the following transformations, using glucose as an example:

Glucose as Electron Donor:

Fermentation of glucose:

$$C_6H_{12}O_6 + 2H_2O = 4H_2 + 2CH_3COOH + 2CO_2 \quad (6)$$

Dehalogenation:

$$CCl_2{=}CCl_2 + 4H_2 = 4HCl + CH_2{=}CH_2 \quad (7)$$

Net reaction:

$$C_6H_{12}O_6 + CCl_2{=}CCl_2 + 2H_2O = 2CH_3COOH + 4HCl + 2CO_2 + CH_2{=}CH_2 \quad (8)$$

Organic compounds that have been added to aquifers to achieve reductive dehalogenation include a variety of soluble organic compounds (pentanol, lactate, methanol, ethanol, molasses, benzoate), materials such as vegetable oils, precipitated compounds such as calcium oleate, natural organic solids such as compost, and various commercially available products, such as "slow hydrogen release compounds" or HRCs. Some of their relative advantages and disadvantages have been addressed [14]. A major limitation for DNAPL dissolution as indicated in Eq. (8) is that not only are four moles of hydrochloric acid produced, but also acetic acid. The latter not only requires additional buffer, but also adds an undesirable organic compound to the aquifer, which can lead to further degradation of water quality through iron, manganese, or sulfate reduction, and methane formation.

There are reports that acetic acid may serve by itself for partial [11] or perhaps complete [6] dehalogenation of PCE and TCE to ethene. This would reduce the concentration of acetic acid, but would lead to the production of two moles of the weak-acid forming carbon dioxide:

$$CCl_2{=}CCl_2 + CH_3COOH + 2H_2O = CH_2{=}CH_2 + 2CO_2 + 4HCl \quad (9)$$

Thus, acetic acid utilization for dehalogenation may have some, but perhaps not a great impact in reducing the acid problem with dehalogenation.

An ideal compound for DNAPL biodegradation would be sufficiently soluble, would not lead to the

production of acetic acid, and would be self-neutralizing of hydrochloric acid production. A compound that meets these characteristics is formate.

Formate as Electron Donor:

Formate disproportionation :

$$4HCOONa + 4H_2O = 4NaHCO_3 + 4H_2 \quad (10)$$

Reductive dehalogenation of PCE :

$$CCl_2{=}CCl_2 + 4H_2 = 4HCl + CH_2{=}CH_2 \quad (11)$$

Acid neutralization :

$$4NaHCO_3 + 4HCl = 4NaCl + 4CO_2 + 4H_2O \quad (12)$$

Net reaction :

$$CCl_2{=}CCl_2 + 4HCOONa = CH_2{=}CH_2 + 4NaCl + 4CO_2 \quad (13)$$

Formate is enzymatically converted into bicarbonate and hydrogen (Eq. (10)). The hydrogen is used for reductive dehalogenation (Eq. (11)), and the hydrochloric acid produced is neutralized by the bicarbonate (Eq. (12)). The net result is the production of ethene, sodium chloride, and carbon dioxide gas. Carbon dioxide is a highly soluble weak-acid gas, and some bicarbonate must be present to buffer its impact.

3. Electron donor, alkalinity, and pH relationships

The rates of biological reactions are affected greatly by pH, with most organisms favoring a near-neutral pH of 7. Thus, as dehalogenation is an acid-producing reaction, sufficient buffer must be present to prevent an adverse drop in pH below neutrality. In groundwaters, pH is generally governed by the carbon dioxide/bicarbonate system. During dehalogenation, the hydrochloric acid produced reacts with bicarbonate to produce carbon dioxide (carbonic acid), which cannot readily escape to the atmosphere, and thus tends to remain in the groundwater.

$$HCl + HCO_3^- = H_2CO_3 + Cl^- \quad (14)$$

The decrease in bicarbonate coupled with increase in carbonic acid both tend to decrease pH according to the relationships:

$$H_2O + CO_2 = H_2CO_3 = H^+ + HCO_3^-,$$
$$\frac{[H^+][HCO_3^-]}{[H_2CO_3]} = K_1 = 4.4\left(10^{-7}\right) \text{ at } 20\,^\circ\text{C} \quad (15)$$

In addition, the acetic acid that is formed when most organics are fermented to form hydrogen also depresses pH:

$$CH_3COOH = H^+ + CH_3COO^-,$$
$$\frac{[H^+][CH_3COO^-]}{[CH_3COOH]} = K_a = 1.72\left(10^{-5}\right) \text{ at } 20\,^\circ\text{C} \quad (16)$$

The pH problem can be especially difficult when applying biological reduction to biologically enhanced dense non-aqueous phase (DNAPL) chlorinated solvent dissolution. Here, dehalogenation of high concentrations of chlorinated solvent can result in production of high hydrochloric acid and acetic acid production, necessitating a large buffer capacity to prevent adverse pH drop. Fig. 1 illustrates the change in pH as a function of the extent of dechlorination with groundwaters starting with different bicarbonate alkalinities (mg/L as $CaCO_3$). This is based upon the use of hydrogen alone for reductive dehalogenation. Enhanced dissolution of TCE or PCE DNAPL might lead to the production of 10 to 20 mM chloride or more. The figure illustrates that in order to maintain pH of about 6.5 or above for maximum reduction rates, no more dechlorination could be achieved than about 3.3 mM with an initial bicarbonate alkalinity of 400 mg/L, double that to 6.6 mM with alkalinity of 800 mg/L, and double that again to 13.2 mM with alkalinity of 1600 mg/L. The later of 13.2 mM chloride could result from complete dechlorination of 4.3 mM TCE, which is only about one half of the TCE solubility of 8.4 mM. Ideally for biologically enhanced dissolution of TCE, much greater dehalogenation than this would be desirable. This

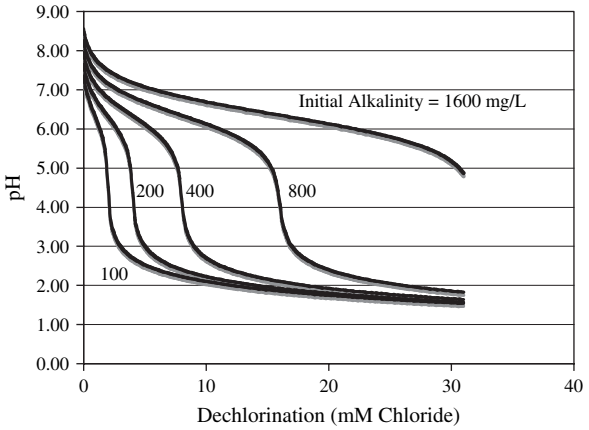

Fig. 1. Relationship between initial bicarbonate alkalinity (mg/L as $CaCO_3$), extent of dechlorination of chlorinated solvents, and resulting pH. The figure assumes hydrogen is used as electron donor with an initial dissolved carbon dioxide concentration of 0.0002 mM and temperature of 20 °C.

would require even higher alkalinity if dehalogenation rates are not to be adversely impacted.

With organic donors that produce acetic acid during fermentation, higher alkalinities are required for a given amount of dehalogenation if the acetic acid cannot be used for dehalogenation. This is illustrated in Fig. 2, where pH changes with the extent of dechlorination are shown for a starting alkalinity of 800 mg/L. In this case, only 3.7 to 4.4 mM chloride dehalogenation would be acceptable while keeping pH of 6.5 or higher with glucose or ethanol, respectively, compared with 6.6 mM chloride with hydrogen. Lactate is a little higher at 5.1 mM because the sodium or other cation associated with lactate forms bicarbonate upon fermentation, thus helping somewhat to maintain pH. Sodium formate on the other hand is much better in this respect, both because of the greater neutralization the sodium provides and also no acetic acid is formed. Here, 10 mM dechlorination can be achieved, or almost three times as much as with glucose. Additionally, if reasonable rates could be obtained with pH down to 6.0, the advantage with formate is even greater as the figure shows.

The impact on pH of other organic compounds can be seen from the summary provided in Table 1. The equations represent the typical relationship between acetic acid formation and hydrogen production during substrate fermentation based upon normal biochemical pathways. The production of both carbonate species and acetic acid from electron donor fermentation, as well as hydrochloric acid from dehalogenation, result in pH reduction, while the presence of sodium ion in carboxylic acid salts increases pH. Compounds for which the sodium balances the acid-forming species, such as formate, have far less impact in lowering pH than those

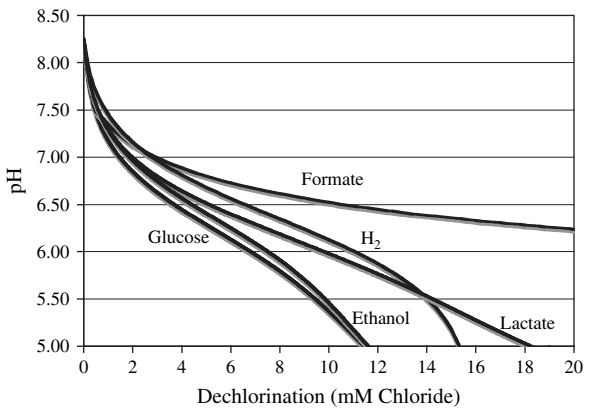

Fig. 2. Effect of extent of chlorinated solvent dechlorination on pH with a starting bicarbonate alkalinity of 800 mg/L (as $CaCO_3$) as a function of electron donor used (other assumptions used are as for Fig. 1).

where the sum of the acid-forming species is greater than the basic impact with sodium. Here, glucose has the greatest effect in lowering pH as illustrated in Fig. 2. Acetate, for which H_2 would not actually be produced from fermentation, is listed at the bottom of the table for comparison as it can be used directly as an electron donor by some organisms capable of dehalogenating PCE and TCE to cDCE.

Fig. 3 illustrates the alkalinity that remains and its forms after 10 mM of dechlorination with different electron donors when starting with 800 mg/L bicarbonate alkalinity. With formate, the total alkalinity remains unchanged. With hydrogen, its decreases directly with the degree of dechlorination. In case of most of the other organic electron donors, except lactate, most of the bicarbonate alkalinity is destroyed and alkalinity due to acetate dominates. In the later cases, measurement of total alkalinity could provide a misleading estimate of the amount of bicarbonate alkalinity available for neutralization of hydrochloric acid from dehalogenation.

The high bicarbonate alkalinity requirement for DNAPL dehalogenation may come in part from alkalinity already present in the aquifer or from chemical addition. The easiest to control compound for alkalinity addition is a bicarbonate such as sodium bicarbonate. Cheaper chemicals such as sodium carbonate or hydroxide would tend to increase the pH too greatly when first added, making pH control more difficult. Use of lime is likely to lead to $CaCO_3$ precipitation and aquifer clogging. Bicarbonate alkalinity may result from solubilization of $CaCO_3$ present in the aquifer, but rates of solubilization tend to be slow at near neutral pH and solubilization requires high CO_2 concentrations, which can be produced from donor fermentation (Table 1). In addition, other reactions such as sulfate and Fe(III) reduction are basic reactions that can result in some bicarbonate alkalinity formation. Thus, the question of how much alkalinity must be added may be somewhat complicated. Nevertheless, the guidance provided from consideration of dechlorination chemistry and reaction stoichiometry can be quite useful in system design and operation.

4. Use of formate as an electron donor

Because of the possible advantage of using formate for reductive dehalogenation of PCE and TCE DNAPL, the question arises as to whether the organisms carrying out reductive dehalogenation of these compounds can actually use formate. While evidence indicates that some dehalogenators that can convert PCE and TCE to cDCE can use formate, it has been reported that the *Dehalococcoides* species that reductively dehalogenate

Table 1
Fermentation equations for dehalogenation by various potential electron donors and the relative amounts of carbonate species, acetate species, and sodium cation involved

Equation	$\Delta(CO_2 + HCO_3^-)$	$\Delta(CH_3COOH + CH_3COO^-)$	Δ (Na^+)
Dehalogenation (Hydrogen Utilization): R–Cl + H_2 = R–H + HCl	0	0	0
Hydrogen Formation from Electron Donor:			
Formate: $HCOONa + H_2O = H_2 + NaHCO_3$	1	0	1
Glucose: 1/4 $C_6H_{12}O_6$ + 1/2 $H_2O = H_2$ + 1/2 CH_3COOH +1/2 CO_2	0.5	0.5	0
Triolean (vegetable oil):	0.021	0.583	0
1/48 $C_{57}H_{104}O_6$ + 13/12 $H_2O = H_2$ + 7/12 CH_3COOH + 1/48 CO_2			
Ethanol: 1/2 CH_3CH_2OH + 1/2 $H_2O = H_2$ + 1/2 CH_3COOH	0	0.5	0
Lactate: 1/2 $CH_3CHOHCOONa + H_2O = H_2$ + 1/2 CH_3COOH + 1/2 $NaHCO_3$	0.5	0.5	0.5
Methanol: $CH_3OH = H_2$ + 1/2 CH_3COOH	0	0.5	0
Propionate: 1/3 $CH_3CH_2COONa + H_2O = H_2$ + 1/3 CH_3COOH + 1/3 $NaHCO_3$	0.33	0.33	0.33
Butyrate: 1/2 $CH_3CH_2CH_2COONa + H_2O = H_2$ + 1/2 CH_3COOH + 1/2 CH_3COONa	0	1	0.5
Acetate: 1/4 CH_3COONa + 3/4 $H_2O = H_2$ + 1/4 $NaHCO_3$ + 1/4 CO_2	0.5	0	0.25

cDCE and VC cannot use formate directly as an electron acceptor [9]. However, this may not be a difficulty in a mixed culture system. In order to explore this, we evaluated the potential of a TCE dehalogenating mixed culture grown on benzoate to use formate for TCE dehalogenation. Here, 12 mL each of a chemostat mixed culture containing *Dehalococcoides* sp. strain VC [4] was added to 25 mL bottles under anaerobic conditions, along with 60 μmol formate and various concentrations of TCE. The changes in TCE, cDCE, VC, ethene, and methane concentration were determined. Fig. 4 indicates that TCE concentrations of 1.5 to 2 mM were somewhat inhibitory to dehalogenation, but with some acclimation, TCE was effectively dehalogenated to cDCE. Methane production occurred readily only in the control culture with no TCE; concentrations of 0.5 mM and above were strongly inhibitory to methane production, as found in previous studies [13]. No transformation beyond cDCE was found in these cultures, which was expected since high TCE and resulting cDCE concentrations were inhibitory to the *Dehalococcoides* strains present. In order to verify this, another study was conducted with VC, rather than TCE, with results shown in Fig. 5. Here, VC dehalogenation occurred about as readily when formate was used as the electron donor

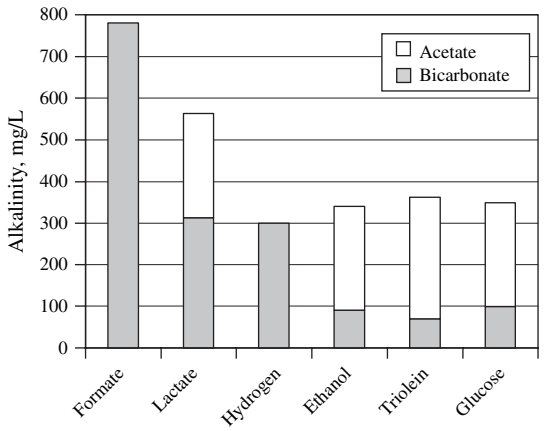

Fig. 3. Final resulting acetate and bicarbonate alkalinity following 10 mM hydrochloric acid formation from chlorinated solvent dehalogenation. The assumed initial bicarbonate alkalinity is 800 mg/L (as $CaCO_3$).

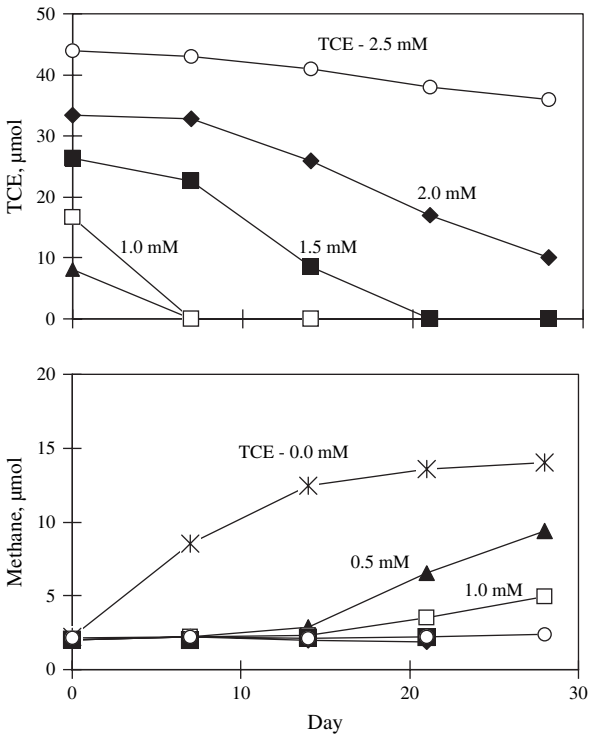

Fig. 4. Dehalogenation of various TCE concentrations and methane production using formate as electron donor with a mixed-culture seed.

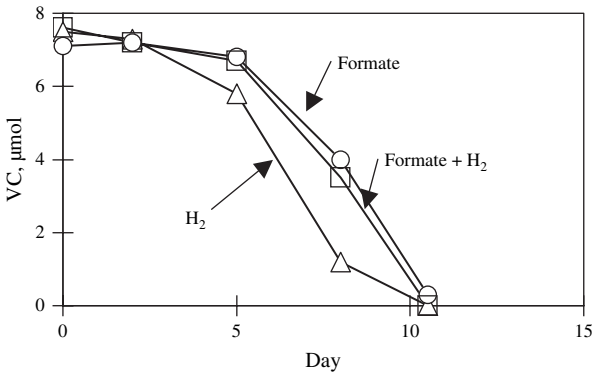

Fig. 5. Dehalogenation of VC using formate or hydrogen as electron donor with a mixed-culture seed.

as when hydrogen was used. These data confirm that in mixed cultures, formate can be used for reductive dehalogenation of VC to ethene. In order for this to be feasible as a method for DNAPL biotransformation, a two-step treatment scheme should be used to achieve complete transformation of PCE or TCE to ethene.

5. Possible treatment schemes

Treatment system design for reductive dehalogenation of PCE and TCE DNAPLs or locations of high chlorinated solvent concentrations need consideration of other factors in addition to donor type, alkalinity requirements, and pH. The advantage of DNAPL instead of more dilute plume dehalogenation is that dehalogenation can be more efficient where concentrations are higher due partly to the better dehalogenation kinetics offered and also to better competition offered against methanogens and other organisms competing for electron donor. In addition, systems can be designed that prevent down-gradient migration of the contaminants. Fig. 6 illustrates three of many possible treatment schemes for reductive dehalogenation of PCE or TCE DNAPL. Fig. 6a represents an extraction injection system [2,7] that can result in the hydraulic isolation of a DNAPL zone while biologically enhanced DNAPL dissolution using formate is carried out. Here, the injection and formate addition are conducted upgradient of the DNAPL zone. The chlorinated compounds present in the extracted water are supplemented with excess formate to inhibit methanogens that would tend to grow near the injection well while at the same time promoting dehalogenation. The excess formate then passes to the DNAPL, where biologically enhanced dissolution by conversion to cDCE occurs. Fig. 6b represents a scheme using two recirculation wells, the first operated in a downflow mode in

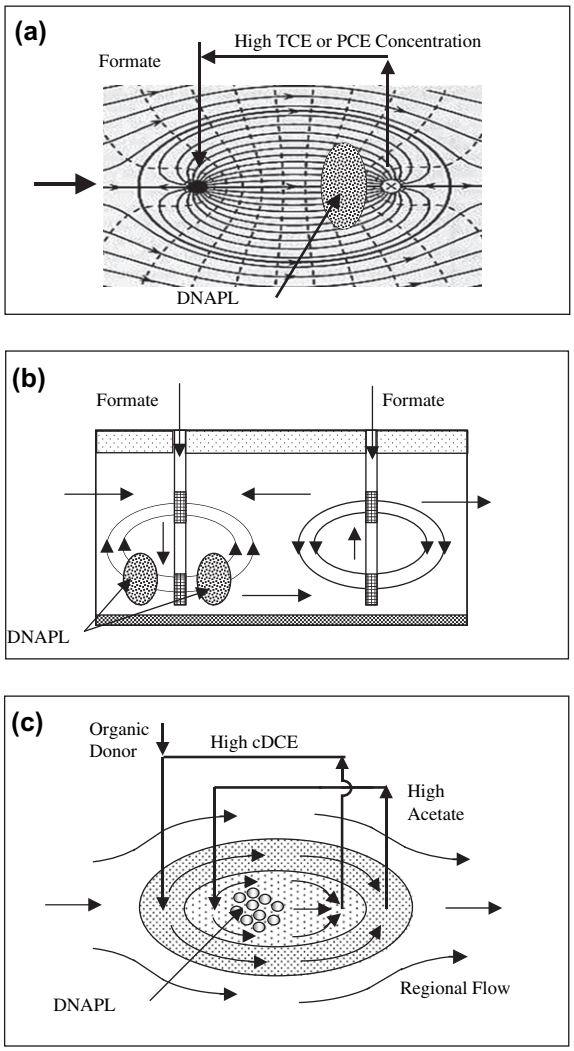

Fig. 6. Possible schemes for biologically enhanced DNAPL dissolution: (a) using extraction and injection wells (plan view); (b) using recirculation wells (vertical view); and (c) using nested wells (plan view).

the DNAPL zone and the second located down gradient and operated in an upflow mode. Such circulation wells have been used successfully for bioremediation of aquifers [10]. At the first circulation well, PCE or TCE is converted primarily to cDCE. The cDCE then flows to the down gradient well where it is dehalogenated to ethene. DNAPL removal is enhanced by either scheme through increase in water movement through the DNAPL zone and by enhanced biological dissolution. Formate permits use of high concentrations as needed to effect rapid DNAPL removal while providing self-buffering to reduce pH control, a problem that would be quite difficult to accomplish with other possible electron donors.

Fig. 6c shows a nested-well system that can be used to achieve improved utilization of other organic electron donors that result in acetic acid as well as hydrogen formation during fermentation. Nested wells have been used successfully for remediation of other contaminants and have the unique ability to isolate source areas of contamination to prevent down-gradient migration [8]. The nested wells include an internal system of injection and extraction wells and an outer system of injection and extraction wells. If injection is carried out at the up-gradient end of groundwater flow, then native groundwater will flow around the nested well system and thus avoid the contamination. As illustrated, electron donor is then added to the injection wells in the external recirculation system. The injected water here also contains a high concentration of cDCE, which helps to prevent growth of competing methanogens near the injection well. The donor is fermented, producing acetic acid and hydrogen, and the dehalogenators use the later for dehalogenation of the cDCE. The acetic acid produced is neutralized to acetate by the bicarbonate alkalinity and withdrawn at the external extraction well. This extracted water containing acetate is then injected into the internal recirculation system where the acetate serves as the donor for conversion of PCE or TCE in the DNAPL to cDCE, which is then removed by the extraction well for injection into the exterior recirculation system. In this manner, both the hydrogen and the acetate from donor fermentation are used. The net overall equation for such conversion using glucose is,

$$1/3C_6H_{12}O_6 + CCl_2{=}CCl_2 + 2H_2O = 4HCl + 2CO_2 + CH_2{=}CH_2 \quad (17)$$

In comparison with Eq. (8) where only the hydrogen produced from glucose fermentation is used for dehalogenation, this treatment system not only reduces the amount of donor needed for complete dehalogenation by two-thirds, but also significantly reduces the amount of bicarbonate buffer needed to maintain near neutral pH by about one third because all acetic acid is used.

Acknowledgments

This study was financially supported by E.I. duPont de Nemours Inc. through the U.S. Environmental Protection Agency sponsored Western Region Hazardous Substance Research Center. Since this manuscript has not been reviewed by these organizations no official endorsement should be inferred.

References

[1] D.T. Adamson, D.Y. Lyon, J.B. Hughes, Flux and product distribution during biological treatment of tetrachloroethene dense non-aqueous-phase liquid, Environmental Science and Technology 38 (2004) 2021–2028.
[2] J.A. Cunningham, M. Reinhard, Injection-extraction treatment well pairs: an alternative to permeable reactive barriers, Ground Water 40 (2002) 599–607.
[3] A.M. Cupples, A.M. Spormann, P.L. McCarty, Growth of a Dehalococcoides-like microorganism on vinyl chloride and *cis*-dichloroethene as electron acceptors as determined by competitive PCR, Applied and Environmental Microbiology 69 (2003) 953–959.
[4] A.M. Cupples, A.M. Spormann, P.L. McCarty, Comparative evaluation of chloroethene dechlorination to ethene by Dehalococcoides-like microorganisms, Environmental Science and Technology 38 (2004) 4768–4774.
[5] J.Z. He, K.M. Ritalahti, K.L. Yang, S.S. Koenigsberg, F.E. Loffler, Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium, Nature 424 (2003) 62–65.
[6] J.Z. He, Y. Sung, M.E. Dollhopf, B.Z. Fathepure, J.M. Tiedje, F.E. Loffler, Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites, Environmental Science & Technology 36 (2002) 3945–3952.
[7] J. Luo, P.K. Kitanidis, Fluid residence times within a recirculation zone created by an extraction-injection well pair, Journal of Hydrology 295 (2004) 149–162.
[8] J. Luo, W. Wu, M.N. Fienen, P.M. Jardine, T.L. Mehlorn, D.B. Watson, O.A. Cirpka, C.S. Criddle, P.K. Kitanidis, A nested-cell approach for in situ remediation, Ground Water 44 (2006) 266–274.
[9] X. Maymo-Gatell, Y.T. Chien, J.M. Gossett, S.H. Zinder, Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene, Science 276 (1997) 1568–1571.
[10] P.L. McCarty, M.N. Goltz, G.D. Hopkins, M.E. Dolan, J.P. Allan, B.T. Kawakami, T.J. Carrothers, Full-scale evaluation of in-situ cometabolic degradation of trichloroethylene in groundwater through toluene injection, Environmental Science & Technology 32 (1998) 88–100.
[11] Y. Sung, K.M. Ritalahti, R.A. Sanford, J.W. Urbance, S.J. Flynn, J.M. Tiedje, F.E. Loffler, Characterization of two tetrachloroethene-reducing, acetate-oxidizing anaerobic bacteria and their description as *Desulfuromonas michiganensis* sp. nov, Applied and Environmental Microbiology 69 (2003) 2964–2974.
[12] K. Yaguchi, T. Suzuki, I. Hirata, T. Itoh, A. Hamada, Biological and chemical characterization of the tetrachloroethylene-degrading bacterium, strain t, Japanese Journal of Toxicology and Environmental Health 40 (1994) 178–184.
[13] Y. Yang, P.L. McCarty, Biologically enhanced dissolution of tetrachloroethene DNAPL, Environmental Science & Technology 34 (2000) 2979–2984.
[14] Y. Yang, P.L. McCarty, Comparison between donor substrates for biologically enhanced tetrachloroethene DNAPL dissolution, Environmental Science & Technology 36 (2002) 3400–3404.