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Comparison between Donor Substrates for Biologically Enhanced Tetrachloroethene DNAPL Dissolution

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Tetrachloroethene (PCE) dense nonaqueous-phase liquid (DNAPL) can act as a persistent groundwater contamination source for decades. Biologically enhanced dissolution of pure PCE DNAPL has potential for reducing DNAPL longevity as indicated previously (*Environ. Sci. Technol.* **2000**, *34*, 2979). Reported here are expanded studies to evaluate donor substrates that offer different remediation strategies for bioenhanced DNAPL dissolution, including pentanol (soluble substrate, fed continuously), calcium oleate (insoluble substrate, placed in column initially by alternate pumping of sodium oleate and calcium chloride), and olive oil (mixed with PCE and placed in column initially). Compared with a no-substrate column control, the DNAPL dissolution rate was enhanced about three times when directly coupled with biological transformation. The major degradation product formed was cDCE, but significant amounts of VC and ethene were also found with some columns. Extensive methanogenesis, which reduced PCE transformation, occurred in both the pentanol-fed and oleate-amended columns, but not in the olive-oil-amended column, suggesting that methanogens managed to colonize column niches where PCE DNAPL was not present. Detrimental methane production in the pentanol-fed column was nearly eliminated by presaturating the feed solution with PCE. These results suggest potential DNAPL remediation strategies to enhance dehalogenation while controlling competitive methanogenic utilization of donor substrates.

Introduction

Because of limited solubility, tetrachloroethene (PCE) spills tend to form dense nonaqueous-phase liquids (DNAPL) in the subsurface, which greatly contribute to long-term contamination of groundwater, posing significant challenges for remediation technologies. Despite previous concerns with potential toxicity of saturated PCE solutions, dehalogenating microbes have been found capable of metabolizing high concentrations of PCE (*1, 2*). Biotransformation through dehalorespiration has been found to result in enhanced DNAPL dissolution rates (*3, 4*) and reduced longevity of the chlorinated ethene components of NAPLs (*5*), thus offering a promising alternative for DNAPL remediation.

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Enhanced DNAPL dissolution through biotransformation can occur because biological degradation can act as a reaction sink near the DNAPL to increase the PCE concentration gradient. In the case of PCE, because its degradation products, trichloroethene (TCE), *cis*-1,2-dichloroethene (cDCE), vinyl chloride (VC), and ethene, all have much higher solubilities than PCE (*3*), PCE dissolution can be greatly enhanced. PCE dehalorespiration requires that a substrate (electron donor) be present or added, and that dehalogenating microorganisms be present. A primary question that needs to be addressed is how to stimulate the desired microbial activity in situ. Compared with bioremediation of contaminated plumes, several factors complicate the situation with PCE DNAPL. First, PCE DNAPL exists as a separate phase, often as nonuniform ganglia, so donor substrate and nutrients must be delivered to near the DNAPL surface to enhance dissolution. Second, microbial growth near the DNAPL can cause a marked reduction in hydraulic conductivity over time, even microbial clogging (see recent review by Baveye et al. (*6*)). Third, gas production and entrapment as a result of microbial activity can result in clogging and flow diversion around DNAPL (*6*−*9*). Methane, resulting from competitive substrate utilization by methanogens, is a concern in reductive dehalogenation, because of both the potential clogging problem and the wastage it represents in donor usage. Fortunately, high concentrations of PCE and its transformation products were found to be highly toxic to methanogens in microcosms so that DNAPL biotransformation that results in such concentrations is expected to reduce this potential problem (*11, 12*). However, different from a microcosm, PCE DNAPL distribution in an aquifer is often spatially heterogeneous and nonuniform, which might provide opportunities for methanogens to grow even when DNAPL is present.

In a previous study on PCE DNAPL dehalogenation using a continuous-flow column with PCE DNAPL present (*3*), we found that PCE DNAPL dissolution rate was improved by about 5-fold when coupled with biological dehalogenation. The major degradation product formed was cDCE, but significant amounts of VC and ethene were also formed. Here we report on comparative studies to evaluate PCE DNAPL dehalogenation with three donor substrates that offer different remediation strategies. The objective was to investigate how different substrates and different substrate delivery methods might impact dehalogenating microbial activity and competing methanogenic activity.

Materials and Methods

Column Construction. Four parallel continuous upflow columns (25-mm i.d. by 300 mm), as listed in Table 1, were constructed to simulate aquifers contaminated with residual PCE DNAPL in a manner similar to that used previously (*3*). Briefly, they were filled with aquifer material that was obtained originally from an uncontaminated groundwater site at Moffett Federal Airfield, Mountain View, California (*15*). During filling, the columns were amended using a 100-μL syringe with about 1 mL of neat PCE in the form of droplets (about 10 μL each) to about 2% saturation of the pore space. The aquifer material did not contain an indigenous population of dehalogenating bacteria, so the columns were bioaugmented by pumping two pore volumes (100 mL, corresponding to about 1 mg of biomass) of anaerobic PCE-dehalogenating culture (*11*) through the columns. This culture was maintained with continuous feed to a closed continuously stirred tank reactor, and was able to achieve nearly complete conversion of PCE to ethene. Then a sterile basal medium containing 20 mg/L of yeast extract (*11*) and

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TABLE 1. Column Feed Substrate and Delivery Methods

column	substrate	substrate delivery	comments
1	none	N/A	experiment control
2	pentanol	continuous feed of 3.7 mM	continuous supply of soluble substrate
3	oleate	pre-fixed as calcium oleate, placed in column initially	occasional supply of insoluble substrate
4	olive oil	premixed with PCE (1:1, v/v), placed in column initially	complete mixing of substrate with PCE DNAPL

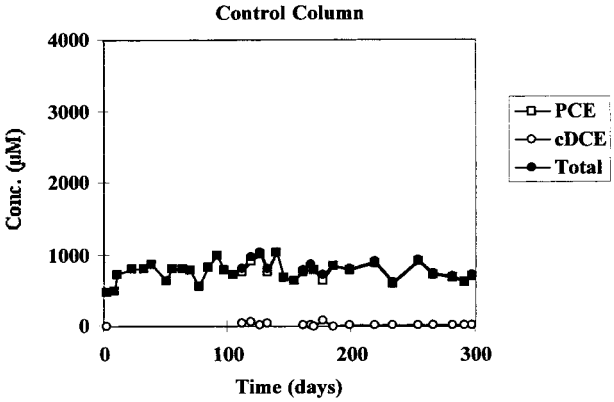

FIGURE 1. PCE and dehalogenation products concentration from control column.

amended with different substrates was continuously fed with a syringe pump at a rate of 3.2 mL/day, resulting in a liquid detention time of about 14 days. Column 1, with basal medium alone, was used as a negative control to monitor PCE dissolution only. For column 2, the basal medium was amended with 3.7 mM of pentanol to represent a soluble substrate. For column 3, sodium oleate (about 1.20 mmol) and calcium chloride (about 12 mmol) were alternatively pumped into the column initially, causing calcium oleate to precipitate in the column, and thus to serve as a slow hydrogen releasing substrate (*12*). Basal medium alone was then pumped through this column over the course of the study. In the 4th column, the 1 mL of neat PCE was not added alone, but was added as a mixture of PCE and olive oil (STAR Originale Olive Oil, Fresco, CA) (1:1, v/v: 1.26 specific gravity) during column construction to investigate the relative effect when substrate and DNAPL are mixed together.

Chemical Analysis and Data Processing. As described previously (*3*), column effluents were collected in 5-mL glass syringes. Gas production resulted in some columns, causing a gaseous phase to be present in the collection syringes. The relative volume of liquid and gas was noted, but only the liquid sample was collected for chlorinated compounds, ethene, and methane analyses as described previously (*3*). By assuming these compounds achieved mass transfer equilibrium between the syringe gas phase and the aqueous phase, the total effluent mass for each compound was calculated using Henry's constants. Chlorinated compounds and ethene concentrations were calculated by dividing the total effluent mass (gas plus liquid) by the effluent liquid volume only.

Results

PCE DNAPL Degradation With Various Substrates. Depicted in Figures 1−4 are the concentration profiles for PCE and its transformation products formed in the effluent over a 300-d period for each column, respectively. Average PCE concentrations in the column effluents during the first 300-d period when stable PCE concentrations were reached are listed in Table 2. The PCE in column 1 effluent (0.76 mM) averaged somewhat less than the aqueous saturation concentration of PCE of 0.9 mM throughout the experimental period. Only

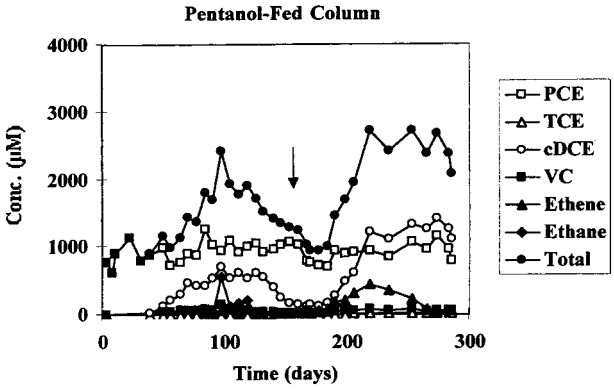

FIGURE 2. PCE and dehalogenation products concentration from pentanol-fed column. Arrow indicates the time when PCE-saturated medium feeding was started.

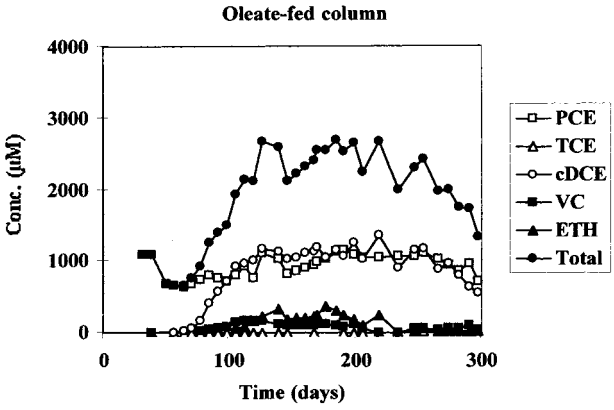

FIGURE 3. PCE and dehalogenation products concentration from oleate-fed column.

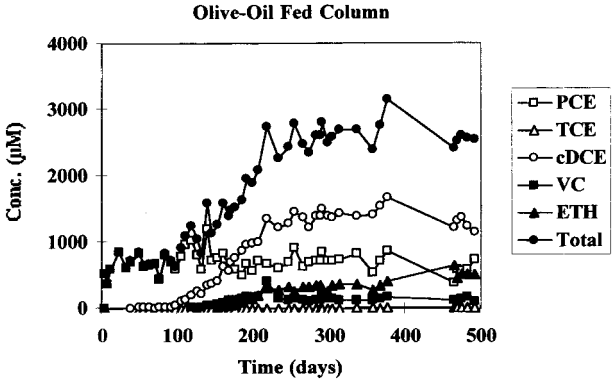

FIGURE 4. PCE and dehalogenation products concentration from olive-oil-fed column.

a trace of cDCE was found here, suggesting that physical dissolution was the main process occurring in the control column. The effluent PCE concentrations in columns 2 (0.93 mM) and 3 (0.89 mM) are statistically higher than that in the control column effluent at the 95% confidence level based upon the *t*-test, being enhanced perhaps by the presence of the substrate, the higher effluent concentration of degrada-

TABLE 2. PCE Concentrations in the Column Effluents

column	time period	no. of samples	effluent PCE concn (μM)[a]
1	day 10−300	35	758 ± 134
2	day 10−300	32	928 ± 143
3	day 40−300	25	887 ± 182
4	day 10−300	38	698 ± 157

[a] Average ± standard deviation.

tion products, bacterial activity, or a combination of these factors. The effluent PCE concentration in column 4 is the lowest among the group as would be expected because the aqueous solubility is reduced when mixed with olive oil. The PCE mole fraction in the equal volume mixture was 0.91. Thus, the effluent PCE concentration from column 4 might be expected to be about 91% of that from column 1 or 690 μM, which is close to the average value measured. Significant dehalogenation occurred in the three substrate-amended columns after an adaptation period. Here, the sum total concentration of PCE and its degradation products greatly exceeded PCE solubility. The increase was by a factor of 2.1 in column 2 before day 150 and 2.5 after that. It increased by a factor of 3 in both columns 3 and 4. These results indicate enhanced dissolution of PCE DNAPL resulted from biological degradation, as found in the previous study (*3*). Column 2 results will be explained subsequently. In column 3, a drop in performance occurred after day 300 (data not shown), which was mainly due to the depletion of the oleate initially added to the column.

A mass balance on substrate and PCE removal over the first 300 days of column operation is provided in Table 3. The estimated amount of substrate removed is based upon the concentration of acetate present in the column effluents, and assumes that acetate was formed as an intermediate product from substrate fermentation and was not consumed in methane fermentation (because acetoclastic methanogens were found to be very sensitive to even low concentrations of chlorinated ethenes (*3*)) or dehalogenation. PCE removal represents the mass of PCE leaving the column in solution as PCE itself or as a daughter product (TCE, cDCE, VC, or ethene). The olive-oil fed column exhibited the greatest conversion of PCE to ethene with effluent ethene concentration near the end of the study of about 0.5 mM. Chloride removal represents the mass of chloride in the effluent calculated to be removed from PCE as represented by the effluent mass of each individual daughter product. On the basis of these mass calculations, the most efficient removal in terms of low substrate mass per unit of PCE or chloride removed is olive oil, followed by pentanol, and then oleate. In all cases, the mass of substrate removed per unit mass of chloride removed in dehalogenation was exceptionally low, varying between 1.24 and 5.31 g substrate/g chloride.

As indicated in Figure 4, dehalogenation continued to occur in the olive oil column for well over a year. Table 4 contains a mass balance for this column for the 493 days of the study. During this time, an estimated 0.22 mmol of olive oil was consumed and 4.96 mmol of chloride was removed through dehalogenation. PCE represented only 35% of the effluent total ethene concentration, whereas ethene itself represented 12.5%. The Table 4 values indicate that the ratio of olive oil used to total ethenes removed was 0.070 mmol/mmol or 0.37 g/g. The ratio of olive oil to chloride removal was 0.044 mmol/mmol or 1.08 g/g. This indicates quite efficient substrate usage resulted for dehalogenation.

A 1-mL aliquot, or 9.8 mmol, of PCE was placed in the columns originally. The Table 4 estimate of 3.1 mmol total ethene removed from the olive oil column represents dissolution of about one-third of the PCE over the 500-day period. Without bioenhanced dissolution, only 11% of the PCE would have been removed in this time period. The extent of PCE transformation is also somewhat underestimated in the Table 4 mass balance because some of the transformation products would partition back into the PCE DNAPL (*4*, *5*) and thus would be held in the column and not counted in the mass balance. The maximum mole fraction of cDCE that might have partitioned back into the DNAPL can be estimated from the cDCE water solubility (36 mM) and the average effluent concentration (1.5 mM), indicating a possible mole fraction in the DNAPL of 1.5/36 or 0.042. With the estimated 6.7 mmol of PCE remaining in the column at the end of the study, this mole fraction would represent 0.042(6.7) mmol or 0.28 mmol cDCE in the DNAPL. This represents 19% of the cDCE mass found in the column effluent. The other transformation product concentrations are significantly smaller relative to their solubilities, and so their partitioning into the DNAPL would have been much lower. Thus, while the estimate of maximum partitioning is significant, it still represents a relatively small percentage of the total transformation that occurred.

Methane Production and Inhibition. Methane production was closely followed after day 100 as shown in Figure 5. Despite the large daily fluctuations, which were probably due to system heterogeneities, significantly different methane production patterns were observed in the three substrate-fed columns. Significant methane was found in column 2 effluent before day 150 and in column 3 continuously. In fact, the methane concentrations were occasionally near or above saturation (1 mM in aqueous phase), with a gas phase generated in the collection syringes and in the columns. This undoubtedly contributed to the frequent clogging of these two columns. In contrast, much less methane was produced in column 4 throughout the study, and clogging was not a problem there.

The fact that significant growth of methanogens occurred in some columns is in contrast with our previous batch culture findings that high concentrations of PCE and its transformation products are inhibitory to methanogens (*3*). The previous batch study dealt with a completely mixed system in which dehalogenating microorganisms, methanogens, and substrates (both PCE and electron donor) were in close proximity to each other so that methanogens were intimately exposed to high concentrations of PCE and its degradation products. It is hypothesized that the heterogeneity of aquifer material in the columns and different spatial distribution of different organisms and substrates might play an important role in the reduced toxicity observed in columns 2 and 3. In these heterogeneous systems, an increasing PCE gradient is likely to exist from the bottom to the top of the column due to PCE dissolution along the water path. Although the effluent PCE concentration was near saturation, the PCE concentration in some portions of the column is likely to be well below saturation. This is especially true near the column inlet. Such low PCE concentration zones can allow the development of methanogenic populations in both the pentanol column, where substrate was fed continuously, and in the oleate column, where the substrate was pre-fixed but not mixed with PCE DNAPL. The observation that methane bubbles gradually developed upward in the pentanol- and oleate-fed columns supports this hypothesis. In the olive-oil-amended column, 4, however, the electron donor and PCE were together. The autotrophic methanogens thus could not obtain substrate without coming into close proximity to DNAPL and toxic solvent concentrations. Although the acetoclastic methanogens would still have a chance to develop, the methane production in this column indicated otherwise. It is very likely that the sensitive acetoclastic methanogens were inhibited by the presence of even a low concentration of chlorinated compounds as observed previously (*3*). In other

TABLE 3. Mass Balance on Substrate and PCE over 300 Days of Column Operation

	substrate, mmol		PCE, mmol		chloride, mmol	removals substrate/PCE		removals substrate/chloride	
column	added	removed	added	removed	removed	mmol/mmol	weight/weight	mmol/mmol	weight/weight
control	0.00	0.00	9.78	0.75	0.03	0.00	0.00	0.00	0.00
pentanol	3.55	1.97	9.78	1.63	1.68	1.21	0.64	1.17	2.91
oleate	1.20	1.27	9.78	1.71	1.94	0.74	1.28	0.65	5.31
olive oil	1.02	0.09	9.78	1.45	1.83	0.06	0.33	0.05	1.24

TABLE 4. Estimated Removals from Olive Oil Column over a 493-Day Period

constituent	removed mmol	percent of total ethene
PCE	1.09	35.0
TCE	0.01	0.4
cDCE	1.45	46.7
VC	0.17	5.4
ETH	0.39	12.5
total ethene	3.10	100.0
organic chloride	4.96	
olive oil	0.22	

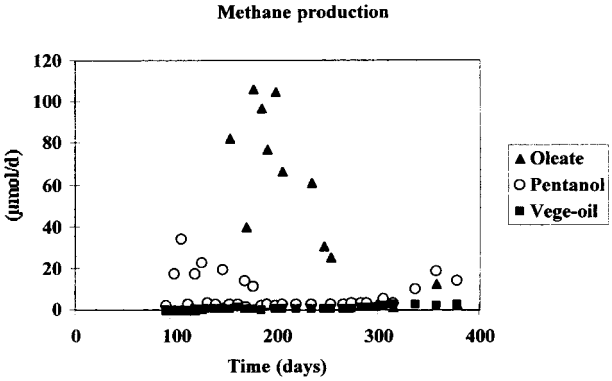

FIGURE 5. Methane production from pentanol-, oleate-, and olive-oil-fed columns.

words, in the dynamic column environment where spatial distribution of substrates and PCE, PCE dissolution, and bacterial activity are constantly interacting with each other, closer association between substrate and PCE appears to help eliminate the growth of methanogenic populations, resulting in improvement of PCE dehalogenation.

The potential importance of close association between substrate and PCE was investigated in the pentanol-fed column. Following day 150,when dehalogenation had essentially stopped (Figure 2), the feed solution was presaturated with PCE so the donor substrate and PCE would move together throughout the column. As shown in Figures 5 and 2, methane production was then greatly reduced, and dehalogenation not only resumed, but increased by 2-fold in terms of PCE DNAPL dissolution. This confirmed that methanogenesis was previously occurring in parts of the column where the PCE concentration was not high.

Discussion

The objective of this study was to further investigate the feasibility of biologically enhanced dissolution of PCE DNAPL. Although some substrates may be relatively inexpensive, excessive use of substrate for dehalogenation results in other kinds of groundwater contamination, such as high dissolved organic carbon concentrations, increased concentrations of soluble iron and manganese, sulfide production, methane production, and aquifer clogging. Concerns have been expressed over whether the substitution of such contamination problems for a problem with chlorinated solvents is truly beneficial (*14*). In previous studies of DNAPL dissolution, we found that less than 1 g of pentanol could be used for dehalogenation of 1 g of PCE to cDCE (*11*). However, it has become common practice to add 100 or more g of donor substrate to achieve dehalogenation of 1 g of chlorinated solvent. One aim of our studies was to seek ways to reduce such wastage.

Several observations here are relevant. First, enhancement of PCE dissolution rate when directly coupled with biological transformation was demonstrated again in this study. The enhancement factor was two to three depending upon the electron donor substrate used. Different from the previous study, in which no PCE was present in the effluent (*3*), near saturation concentrations of PCE were detected in the effluents of all four columns (Figures 1−4). This is due to the different experimental designs used in the two studies. In the previous study, the top $^1/_3$ of the pentanol-fed column contained no aquifer material and was filled with culture, thus PCE emerging from the aquifer material was dehalogenated there without further opportunity for replacement by dissolution. Another difference is in the dissolution enhancement factor which was comparatively lower here than with the previous enhancement factor found of about 5. This might be due to the difference in flow rates used. The only other significant operational difference was that the previous column had a 40% higher velocity through the aquifer-material-filled section. How this might have increased the dissolution rate is not clear.

Second, because of the spatial heterogeneity of the column system, methanogens were found to be active competitors with dehalogenating organisms for substrate despite the expected toxicity of PCE DNAPL to the methanogens (*3*). Methanogens are detrimental not only because a significant amount of substrate is wasted for a nonuseful process, but significant biomass and methane gas production may result in clogging and thus isolating the DNAPL from the substrate. With the soluble substrate pentanol feed, methanogenic competition for substrate was the major problem as they consumed the pentanol before it reached the DNAPL area. With oleate, methane clogging of the aquifer was the major problem as this prevented flow of water past the DNAPL to carry away the dissolution products. Approaches that act to inhibit the activity of methanogens are thus to be favored.

The results of this study provide a comparison between different substrate and delivery strategies that might be attempted for enhanced PCE DNAPL dissolution. Continuous delivery of the soluble substrate pentanol resulted in active dehalogenation and significant enhancement of DNAPL dissolution initially, but this enhancement was difficult to maintain. First, an increase in the dehalogenating microbial populations around the PCE DNAPL ganglia can reduce the diffusion flux of donor substrate to where needed, and second, the subsequent growth of competing methanogens eventually results in their becoming dominant in the system and capturing the majority of the substrate as well as producing methane gas that clogs the system. This dominance occurs

because the methanogens can grow in niches away from the DNAPL, thus avoiding toxicity problems. Possible options for this problem are to provide a pulse supply of substrate instead of continuous supply to control biomass accumulation, and, as found here, to inhibit methanogens by adding donor to extracted groundwater containing a significant amount of PCE or other toxic dehalogenation products prior to injection. Unfortunately, this will also result in decreased net enhancement of PCE dissolution.

Batch delivery of insoluble substrates such as calcium oleate can also stimulate methanogens, but in this case, both dehalogenators and methanogens were more able to share the substrate because it is essentially fixed in place until used. Substrate near PCE ganglia would be used primarily by dehalogenators because of PCE toxicity there, but methanogens could use the oleate that was not near DNAPL. This approach has an advantage in operational cost as reinjection of oleate needs be done infrequently. On the basis of the column studies twice per year may be adequate, although field studies are required to verify this. A disadvantage is that some methane production still occurs, which can cause undesired clogging of the aquifer.

The ideal approach found here is to have the DNAPL mixed with another NAPL such as olive oil as illustrated by the results from column 4. Because the substrate here remains close to the DNAPL, growth of dehalogenators is favored, and conditions suitable for methanogens do not exist. As a result, efficient usage of substrate for dehalogenation alone resulted, and clogging did not become a significant problem. Olive oil is about 84% oleic acid, and so column 4 has biochemical pathway similarities to column 3 that contained oleate. The advantage of having the substrate mixed with DNAPL as in column 4, rather than distributed more randomly throughout the aquifer material as in column 3, is apparent.

Mixed PCE DNAPL and donor substrate NAPL often exists at contaminated sites, such as those where petroleum products and chlorinated solvents have been disposed together. Another example is contamination resulting from leaking tanks containing mixtures of waste solvents such as mixtures of acetone, xylene, or methyl ethyl ketone with chlorinated solvents. Such disposal is no doubt responsible for many of the common observations of intrinsic chlorinated solvent dehalogenation. The question arises as to how such a mixture could be created with existing PCE DNAPL contamination. One approach worth consideration is the introduction into the aquifer of a water solution containing a donor substrate that partitions readily into DNAPL. When the water solution passes by DNAPL ganglia, then the substrate would partition out of the water and into the DNAPL. This was one of the major reasons for our selection of pentanol as a donor substrate, although it does not partition adequately. Another advantage of pentanol is that its fermentation leads to a high production of hydrogen relative to acetate, a ratio of 5 to 2. However, the partition coefficient for pentanol was found to be not high enough to be effective at partitioning. Longer chain alcohols were found to have higher partition coefficients, but lower water solubility, and so the desired effect was difficult to achieve here as well. Another possibility is to use a surface active agent that has a high water solubility, but also can sorb strongly to the surface of DNAPL. Use of high-concentration solutions of sodium or potassium oleate, but without calcium chloride precipitation as used here, is one possibility. Other approaches in this direction may be worth exploring.

Acknowledgments

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