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research bulletin

CL:AIRE site bulletins provide a source of background information on contaminated sites which have been used within the scope of CL:AIRE technology demonstration and research projects. This bulletin describes the first results from Project SABRE, and focuses on a laboratory microcosm study.

Project SABRE (Source Area BioREmediation) Progress Report: Results of a Laboratory Microcosm Study to Determine the Potential for Bioremediation of Chlorinated Solvent DNAPL Source Areas

1. BACKGROUND

Chlorinated solvents have been used in large quantities by a diverse range of industries including chemicals production, metalworking, automotive, aerospace, electronics and dry cleaning. They account for approximately 30% of groundwater pollution incidents in England and Wales with trichloroethene (TCE) reported to be the most frequent contaminant within this group (Environment Agency, 1996).

In the subsurface, all except very small releases of chlorinated solvents may result in a source area that contains dense non-aqueous phase liquid (DNAPL) contaminant. Such sources will persist for decades and act as long-term sources of groundwater contamination (Environment Agency, 2003) but current methods of remediation are usually very expensive and of uncertain result (National Research Council, 2004).

Under anaerobic conditions, dehalorespiring bacteria (e.g., *Dehalococcoides ethenogenes*; DHE) use chlorinated ethenes as terminal electron acceptors for respiration. This metabolism involves a step-wise removal of chlorine atoms from the molecule, ultimately to yield ethene ("reductive dechlorination"). This degradation process has been shown to be viable for field-scale bioremediation of dissolved chlorinated solvent plumes (e.g. Ellis et al., 2000; Major et al., 2002) and laboratory studies have suggested that it is effective in the presence of chlorinated solvent DNAPL (e.g. Maymo-Gatell et al., 1997; Yang and McCarty, 2000; 2002). Indeed degradation may occur more efficiently, since the growth of competing microorganisms is inhibited in the presence of DNAPL (Cope & Hughes, 2001).

Project SABRE (Source Area BioREmediation) is a collaborative project being undertaken by a multidisciplinary team from the UK, USA and Canada, supported through the DTI Bioremediation LINK programme. The objective is to develop and demonstrate quantitatively, in a scientifically robust manner through laboratory, field and numerical assessment, that *in situ* enhanced anaerobic bioremediation can result in cost-effective treatment of chlorinated solvent DNAPL source areas. Project SABRE commenced in October 2004 and will run until the end of 2008. A site in The Midlands of England hosts the SABRE fieldwork.

This is the first in a series of CL:AIRE Research Bulletins that will report results arising during the programme.

Figure 1. Picture of microcosm bottles.

2. INTRODUCTION

A key activity in Project SABRE is the execution of laboratory studies to determine the optimal conditions and bioremediation treatment regime to achieve the objectives of the field portion of the project. The laboratory programme involves both batch microcosm and continuous flow column studies, which provide essential input data to the design and operation of the field test and will support the interpretation of the results obtained in the field.

The first stage in the laboratory programme was a large-scale, multi-laboratory batch microcosm study to determine the optimal electron donor (carbon and energy source), supplemental nutrient, and bioaugmentation combination to support reductive dechlorination of TCE in site soil and groundwater. A unique feature of the SABRE study was the consideration of slow release and partitioning electron donors such as soya bean oil, hexanol, and butyl acetate. These donors have the potential to partition onto the soil and into the TCE DNAPL to provide a long-term source of energy for the reductive dechlorination process.

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3. EXPERIMENTAL SET-UP AND DESIGN

Microcosm studies were performed in sterile 250 millilitre (mL) screw-cap bottles containing 100 grams of soil and 180 mL of groundwater from the site (Figure 1). A fractional factorial, statistically based experimental design was used to test six different electron donors (sodium lactate, magnesium acetate, methanol, SRS™ (a commercial form of emulsified soya bean oil provided by Terra Systems Inc.), 1-hexanol, and n-butyl-acetate), supplemental nutrient addition in the form of diammonium phosphate and yeast extract, bioaugmentation (using KB-1 bacteria provided by SiREM), and two concentrations of TCE (100 and 400 mg/L) (Figure 2). Unamended and killed controls were used to measure intrinsic biodegradation and to evaluate abiotic losses from the bottles.

#	Sample	Donor	Bioaugment	[TCE]	Nutrients
1	1A	Lactate*	No	High	Yes
2	1B	Lactate*	No	High	Yes
3	2C	Lactate*	No	Low	No
4	3C	Lactate*	Yes	High	No
5	4A	Lactate*	Yes	Low	Yes
6	4B	Lactate*	Yes	Low	Yes
7	5A	Acetate*	No	High	Yes
8	5B	Acetate*	No	High	Yes
9	6C	Acetate*	No	Low	No
10	7C	Acetate*	Yes	High	No
11	8A	Acetate*	Yes	Low	Yes
12	8B	Acetate*	Yes	Low	Yes
13	9A	Methanol*	No	High	Yes
14	9B	Methanol*	No	High	Yes
15	10C	Methanol*	No	Low	No
16	11C	Methanol*	Yes	High	No
17	12A	Methanol*	Yes	Low	Yes
18	12B	Methanol*	Yes	Low	Yes
19	13A	Soybean Oil	No	High	Yes
20	13B	Soybean Oil	No	High	Yes
21	14C	Soybean Oil	No	Low	No
22	15C	Soybean Oil	Yes	High	No
23	16A	Soybean Oil	Yes	Low	Yes
24	16B	Soybean Oil	Yes	Low	Yes
25	17A	Hexanol	No	High	Yes
26	17B	Hexanol	No	High	Yes
27	18C	Hexanol	No	Low	No
28	19C	Hexanol	Yes	High	No
29	20A	Hexanol	Yes	Low	Yes
30	20B	Hexanol	Yes	Low	Yes
31	21A	Butyl Acetate	No	High	Yes
32	21B	Butyl Acetate	No	High	Yes
33	22C	Butyl Acetate	No	Low	No
34	23C	Butyl Acetate	Yes	High	No
35	24A	Butyl Acetate	Yes	Low	Yes
36	24B	Butyl Acetate	Yes	Low	Yes
37	25C	Unamended Control	No	Low	No
38	26A	Unamended Control	No	High	No
39	26B	Unamended Control	No	High	No
40	27A	Killed Control	No	Low	No
41	27B	Killed Control	No	Low	No
42	28C	Killed Control	No	High	No

* = weekly donor additions

Figure 2. Example microcosm treatments for one laboratory.

Four industrial laboratories (DuPont, GE, SiREM, and Terra Systems) performed the microcosm study. Microcosm treatments were set up in triplicate, with replicates divided among the laboratories. The bottles were incubated in the dark at 20-22 ºC. Including controls, the study initially consisted of 168 bottles. Nine bottles were subsequently added due to a bioaugmentation error, bringing the final total to 177 bottles (153 experimental bottles and 24 unamended and killed controls).

The amount of electron donor added to each bottle was calculated based upon the stoichiometric demand of the primary contaminant and the background demand imposed by competitive electron acceptor processes such as nitrate reduction, iron reduction, sulphate reduction, and methanogenesis. Sulphate was present in the groundwater at 1250 mg/L, making sulphate reduction the major electron accepting process (the end-point of respiration) in the study. An engineering safety factor of 3x was used to account for competitive processes that could not be easily quantified. The soluble donors were added at every two weeks throughout the study while the slow release and partitioning donors were added only at the beginning of the study.

Volatile organic compound (VOC) analyses were performed at two to three week intervals throughout the study. Each experimental variable (donor, supplemental nutrients, bioaugmentation, and TCE level) was carefully evaluated for its contribution to promoting complete reductive dechlorination of TCE to ethene using analysis of variance (ANOVA) and survival analysis using a Weibull regression model. The key metric measured in the study was the time required to reduce 98% of the VOCs in each bottle to ethene. The main microcosm experiment lasted 203 days.

In addition to VOC analysis, other indicators of biological activity were also monitored during the study. These included periodic measurements of pH, sulphate, and gas production (e.g. to measure methanogenesis). Multiple techniques were used to characterize the microbial community structure both initially and after introduction of electron donor, nutrients, and KB-1 bacteria into the microcosm bottles. These techniques included semi-quantitative polymerase chain reaction (PCR) to enumerate *Dehalococcoides ethenogenes*, denaturing gradient gel electrophoresis (DGGE) to qualitatively assess the community composition, and phospholipid fatty acid analysis (PLFA) to measure total biomass.

4. RESULTS

All electron donors promoted complete dechlorination of TCE to ethene in at least some bottles during the course of the study. The classical reductive dechlorination sequence of TCE to cis-1,2-dichloroethene (c-DCE) to vinyl chloride (VC) to ethene was observed in all cases (Figure 3). Molar balances were generally excellent in both the experimental bottles and the controls, indicating that abiotic losses were minimal.

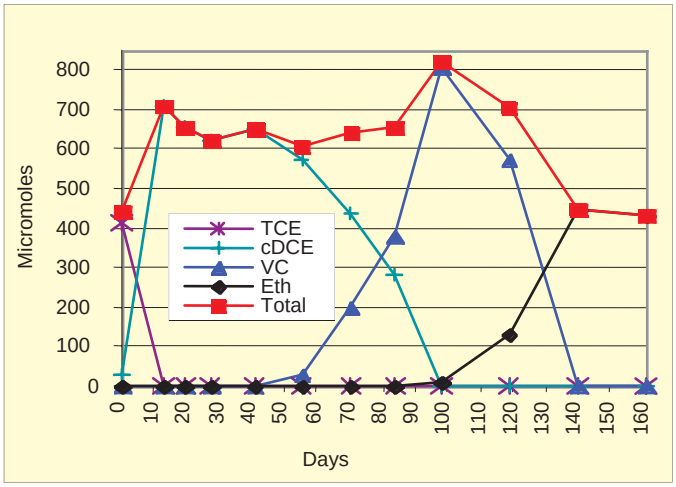

Figure 3. Example of the progression of dechlorination in a soya bean oil amended bottle. The bottle contained the high (400 mg/L) level of TCE, was amended with nutrients, but was not bioaugmented. Time to complete dechlorination was around 140 days. The molar balance is shown in red.

121 out of 153 experimental bottles either reached complete dechlorination to ethene during the 203 days of the study or progressed far enough so that the time to completion could be estimated using a simple logistic model. When these results were analysed using ANOVA, all the key variables in the experiment (electron donor, bioaugmentation, nutrients, laboratory, and TCE concentration) had a statistically significant influence on the time to complete dechlorination at a 95% confidence level (Table 1). Several two-way interaction terms were also statistically significant, but will not be discussed here.

Various views of the composite data are shown in Figures 4-6. Electron donors are compared in Figure 4. Here, soya bean oil is shown to promote the fastest dechlorination of TCE to ethene at low TCE concentrations and was the only donor in which all bottles reached completion at high TCE concentrations. Bioaugmentation and nutrient addition are compared in Figures 5 and 6. In both cases these amendments substantially reduced the time required to achieve the complete dechlorination of TCE to ethene.

Table 1: Analysis of variance corresponding to the linear model measuring the time to complete dechlorination (log $D_{0.02}$).

RESPONSE: log($D_{0.02}$)

ANOVA TABLE (TYPE II TESTS)

FACTOR	SS	DF	*F*	*p*
Electron	3.06	5	10.23	< 0.001
Bio	3.08	1	51.43	< 0.001
Nutrients	4.56	1	76.10	< 0.001
Lab	3.46	3	19.26	< 0.001
TCE	0.15	1	2.54	0.11
TCE_0	0.27	1	4.52	0.036
Electron:Bio	0.85	5	2.83	0.020
Electron:Nutrients	1.19	5	3.96	0.0027
Electron:TCE	2.16	5	7.21	< 0.001
Bio:Nutrients	0.24	1	3.97	0.049
Bio:TCE	2.7×10^{-4}	1	4.56×10^{-3}	0.95
Nutrients:TCE	0.06	1	0.98	0.33
Residuals	5.39	90		

Note - Electron denotes electron donor and Bio denotes bioaugmentation. All the factors are categorical, except TCE_0, which is quantitative, and measures the initial number of micromoles of TCE. The notation "Electron:Bio'", for example, denotes a two-factor interaction.

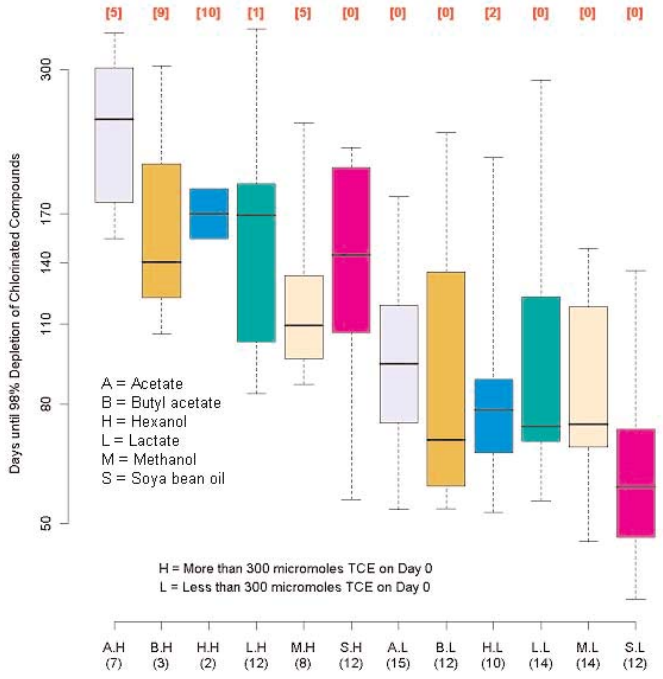

Figure 4. Boxplots summarize the time to completion for experimental bottles as a function of electron donor. Data is grouped by high and low TCE concentrations. The dark line through each box is the median value. The boxes represent one standard deviation of the data. All data fall within the whiskers. The number of bottles in each donor group appear in black along the x-axis. The number of bottles where dechlorination had not yet progressed sufficiently to allow estimation of a completion time appear in red at the top. Electron donor effects shown here are confounded with the effects of bioaugmentation, nutrients, and laboratory.

The ANOVA was limited by the fact that bottles where the time to completion could not be accurately determined were not part of the analysis, thus biasing the analysis in the favour of the under-performing treatments. To remedy this inequity, a survival analysis was performed using a Weibull regression model to incorporate all the data. Models of this type are commonly used to perform lifetime studies (for example, in studies of survival of leukaemia patient as a function of white blood counts and other physiological attributes).

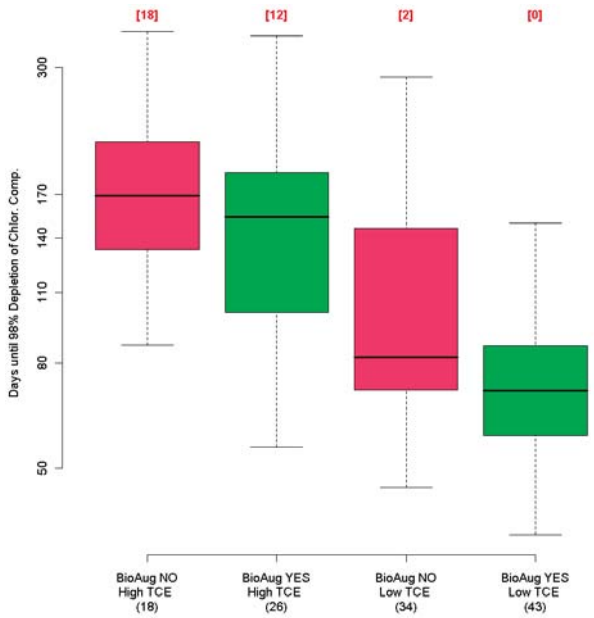

Figure 5. Boxplots summarize the time to completion for experimental bottles as a function of bioaugmentation. Data is grouped by high and low TCE concentrations. The dark line through each box is the median value. The boxes represent one standard deviation of the data. All data fall within the whiskers. The number of bottles in each group appear in black along the x-axis. The number of bottles where dechlorination had not yet progressed sufficiently to allow estimation of a completion time appear in red at the top. Bioaugmentation effects shown here are confounded with the effects of electron donor, nutrients, and laboratory.

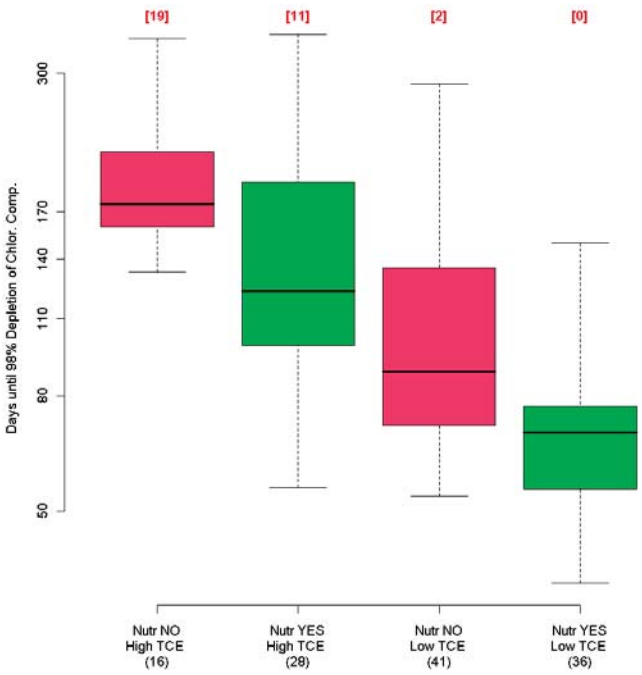

Figure 6. Boxplots summarize the time to completion for experimental bottles as a function of nutrient addition. Data is grouped by high and low TCE concentrations. The dark line through each box is the median value. The boxes represent one standard deviation of the data. All data fall within the whiskers. The number of bottles in each donor group appear in black along the x-axis. The number of bottles where dechlorination had not yet progressed sufficiently to allow estimation of a completion time appear in red at the top. Nutrient effects shown here are confounded with the effects of bioaugmentation, nutrients, and laboratory.

When this analysis was applied to the data, soya bean oil was shown to be the best electron donor, reducing the time to completion by 1.6 times over acetate, an arbitrarily chosen reference point (Table 2). However, soya bean oil could not be statistically differentiated from methanol, which was 1.4 times faster than acetate, or lactate, which was 1.2 times faster. By similar analyses, bioaugmentation reduced the time to completion by 1.9 times over the non-bioaugmented case, while nutrient addition reduced the time to completion by 2.0 times. These effects were both statistically significant.

Table 2: Differential effects of the electron donors, bioaugmentation, and nutrients as determined by survival analysis using a Weibull regression model.

HIGH AND LOW INITIAL TCE ENVIRONMENTS SEPARATE SCALE ESTIMATES FOR LOW AND HIGH INITIAL TCE SUBSETS							
	ELECTRON DONOR					BIOAUGMENTATION	NUTRIENTS
	B/A	H/A	L/A	M/A	S/A	Y/N	Y/N
	0.87	**0.55**	**1.2**	**1.4**	**1.6**	**1.9**	**2.0**
U	1.1	0.71	1.6	1.8	2.1	2.4	2.5
L	0.68	0.42	0.98	1.1	1.3	1.5	1.6

Notes - B = Butyl Acetate, H = Hexanol, L = Lactate, M = Methanol, and S = Soya bean Oil, all relative to A = Acetate. The last two lines of the table, labelled ``U'' and ``L'', list the upper and lower end-points of approximate 95% confidence intervals for the differential effects, hence convey a measure of the uncertainty affecting the corresponding estimates.

Twenty-seven bottles that went to completion mid-way through the main experiment were subsequently re-spiked with 800 mg/L TCE to evaluate process performance under higher TCE loadings. More than half the bottles are showing dechlorination of TCE to c-DCE and VC after 180 days. A few bottles are now completely at ethene. Soya bean oil again appears to be superior to the other donors in promoting reductive dechlorination. However, statistical analysis of these results is not yet complete.

5. CONCLUSIONS

The results of this study indicate that reductive dechlorination of TCE to ethene is feasible at the very high TCE concentrations that will be encountered in a DNAPL source area. The results indicate that emulsified soya bean oil supported the fastest dechlorination of TCE to ethene, but was not statistically significantly better than methanol or lactate. Both bioaugmentation and nutrient addition had statistically significant beneficial effects on the speed of reductive dechlorination. Soya bean oil is a slow release, long-lasting electron donor that will only need to be added intermittently in the field, giving it substantial operational advantages over both lactate and methanol. Therefore, based upon these results, soya bean oil, bioaugmentation, and nutrient addition will all be carried forward into the field component of the SABRE programme.

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Further information about this project may be obtained by visiting the SABRE website (www.claire.co.uk/sabre.php) or by contacting:

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