CL:AIRE

SAB 3
(September 2010)

sabre bulletin

CL:AIRE SABRE bulletins describe specific, practical aspects of research from the LINK Bioremediation Project SABRE, which aimed to develop and demonstrate the effectiveness of *in situ* enhanced anaerobic bioremediation for the treatment of chlorinated solvent DNAPL source areas. This bulletin describes laboratory-based continuous flow column studies that were used in the design and operation of the field work and to interpret results obtained from the field.

Results of Laboratory Column Studies to Determine the Potential for Bioremediation of Chlorinated Solvent DNAPL Source Areas

1. BACKGROUND

Tetrachloroethene (PCE) and trichloroethene (TCE) are chlorinated solvents that have been used extensively in industry and are common groundwater contaminants. The limited water solubility of PCE and TCE often means that PCE and TCE contaminated sites contain dense non-aqueous phase liquid (DNAPL) source zones that provide a long term source of groundwater contamination (Johnson and Pankow, 1992). Under anaerobic conditions, PCE, TCE, and other chlorinated ethenes may be biodegraded via reductive dechlorination (biologically mediated step-wise removal of chlorine atoms) to form ethene. This microbial activity is commonly used in the treatment of dissolved-phase TCE plumes. However, reductive dechlorination has also been observed at PCE and TCE concentrations associated with the presence of DNAPL, typically ≥10% aqueous solubility (Harkness et al., 1999; Nielsen and Keasling, 1999). Additionally, in the microcosm phase of the SABRE project TCE concentrations as high as 800 mg/L were dechlorinated to cis-1,2 dichloroethene (cDCE) and vinyl chloride (VC) and in a few cases completely to ethene (reported previously in CL:AIRE Bulletin RB6). These observations indicate that reductive dechlorination may be a viable *in situ* treatment for TCE DNAPL at the SABRE site.

The primary goal of bioenhanced DNAPL treatment is to achieve and maintain a high flux of contaminant from the DNAPL and sorbed phases to the aqueous phase where complete dechlorination to ethene can occur (Aulenta et al., 2006). Increasing the flux over dissolution alone shortens the lifetime of the DNAPL thereby decreasing the remediation time required. In reductive dechlorination, chlorinated ethenes are used as electron acceptors (something to breathe) and fermentable substrates (ultimately hydrogen) are used as electron donors (something to eat) by the dechlorinating bacteria. Addition of electron donors that partition into the DNAPL phase and then slowly dissolve back into the water phase have been shown in laboratory studies to provide optimum conditions for dechlorination to occur at the DNAPL-water interface and enhance dissolution (Yang and McCarty, 2002). Emulsified vegetable oil (EVO) is an electron donor widely used in treatment of dissolved phase TCE and also has the potential to partition into the DNAPL phase (AFCEE, 2007).

Project SABRE (Source Area BioREmediation) was a collaborative project undertaken by a multidisciplinary team from the UK, USA, and Canada, supported through the DTI Bioremediation LINK programme. The objective was to develop and demonstrate that *in situ* enhanced anaerobic bioremediation can result in effective treatment of chlorinated solvent DNAPL source areas. An important aspect of the SABRE programme was the field application of DNAPL-partitioning electron donors to the source zone to provide a source of electron donor at the DNAPL-water interface. Project SABRE commenced in October 2004 and ran through 2009. A site in the Midlands of England hosts the SABRE fieldwork. This bulletin is one in a series of CL:AIRE SABRE Bulletins that report results arising during the programme.

2. INTRODUCTION

A key component of Project SABRE was laboratory studies to select bioremediation amendments to be applied to the field portion of the project. The laboratory programme has included both batch microcosm and continuous flow column studies that were used in the design and operation of the field test and which will support the interpretation of results obtained from the field. This report focuses on results from the continuous flow columns.

Earlier batch study microcosm experiments demonstrated that EVO (for the SABRE project, TerraSystems product, SRS™ was employed) was among the most effective donors in supporting biodegradation of high concentrations of TCE and that bioaugmentation (using SiREM's KB-1®) and nutrient addition (diammonium phosphate) were also beneficial to the process (CL:AIRE Research Bulletin 6, 2006).

The column studies were carried out in three different industry laboratories (General Electric (GE), SiREM, and DuPont), each team addressing a different objective as follows:

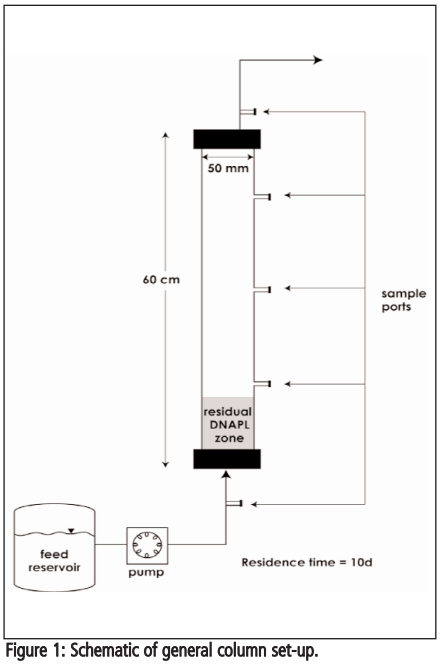

Figure 1: Schematic of general column set-up.

1) Understand the physical-chemical behaviour of partitioning donors in flow systems (GE);
2) Estimate the extent of DNAPL dissolution enhancement created by the bioremediation process (SiREM);
3) Determine the effects of sulphate on dechlorination (DuPont); and
4) Develop kinetic data to support process modelling (All).

The results from these column studies have been collected into two areas: data and observations where a DNAPL source zone is present in the columns (DNAPL phase), and data and observations where high concentrations of TCE exist in the aqueous phase, representative of an area down gradient from a DNAPL source zone (plume phase).

3. EXPERIMENTAL SETUP AND DESIGN

All laboratories used soil collected from the site and artificial groundwater (AGW) that was designed to match the site groundwater chemistry. A schematic diagram of the experimental systems is shown in Figure 1. Each laboratory study addressed a different objective and therefore each used a unique experimental setup and design as shown in Table 1.

Column effluents were sampled on a twice a week to weekly basis. Intermediate port samples were collected from the GE and DuPont columns approximately monthly. Samples collected were analysed for volatile organic compounds (VOCs), dissolved hydrocarbon gases (DHGs), anions, and pH. In addition to these analyses, and on a less frequent basis, samples were collected for quantitative polymerase chain reaction

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Table 1: Column operating conditions.

	SiREM		GE		Dupont	
	DNAPL Active Control	DNAPL EVO amended	Plume EVO amended	DNAPL EVO amended	Plume + Sulphate EVO amended	Plume Sulphate free EVO amended
Column Dimensions	60 cm X 7.5 cm	60 cm X 7.5 cm	60 cm X 5.0 cm	60 cm X 5.0 cm	2-60 cm X 7.5 cm connected to form one 120 cm column	2-60 cm X 7.5 cm connected to form one 120 cm column
Flow rate (mL/min)	0.07	0.07	0.04	0.04	0.07	0.08
Residence Time (days)	6.0	6.0	6.0	6.0	45	45
DNAPL Retained (g)	90.7	31.5	NA	75	NA	NA
Approximate residual saturation of DNAPL (%)	20	10	NA	20	NA	NA
TCE concentration in AGW (mg/L)	Not added	300 (plume phase, starting at day 329)	250	250	400	400
EVO (Terra Systems, SRS™)	Not added	42 g (Day 47)	1st addition - 14 g (Day 63) 2nd addition (columns connected) - 12 g (Day 375)	1st addition - 14 g (Day 63) 2nd addition - 14 g (Day 105)	1st addition - 17 g (Day 133) 2nd addition - Target 17 g (Day 446)	1st addition - 4.5 g (Day 141) 2nd addition - Target 17 g (Day 446)
Nutrients (diammonium phosphate) (mg/L)	Not added	25 (added continuously starting at day 125)	25 (added continuously starting at day 205)	25 (added continuously starting at day 205)	Not added	Not added
KB-1® (mL)	Not added	10 mL (added on day 78)	NA	4.0 mL (added on day 202 at 1 mL/hr)	10 mL (added on day 183)	10 mL (added on day 183)
pH adjusted	No	Yes (starting at day 246)	Yes (starting at day 523) columns connected	Yes (starting at day 523) columns connected	No	No
Columns connected	No	No	Yes from DNAPL column on day 380	Yes, to control column on Day 380	No	No

Notes: AGW - artificial groundwater; cm - centimetres; EVO - emulsified vegetable oil; g - grams; mL/min - millilitres per minute; mg/L - milligrams per litre; mL - millilitres; NA - not applicable; % - percent

(qPCR) to enumerate *Dehalococcoides* (Dhc) and the vinyl chloride reductase gene (vcrA). These analyses are important to monitor in bioremediation studies because Dhc are the only bacteria known to completely dechlorinate chlorinated ethenes to ethene. Certain strains of Dhc contain the vcrA gene, which is required for efficient conversion of VC to ethene.

4. RESULTS

4.1 DNAPL Phase Results

The GE column study consisted of two columns, one with a TCE DNAPL source zone and one without. The study explored the interaction of the TCE DNAPL and EVO. A comparison of the total organic carbon (TOC) mass balance in the influent and effluent of the GE columns showed that the column with the DNAPL source zone retained 26% more EVO than the column without a DNAPL source zone (Table 2), indicating that the EVO partitioned into the DNAPL. Although bioaugmented with the dechlorinating culture KB-1®, the DNAPL EVO amended column did not show significant TCE dechlorination, producing only a small amount of cDCE (circa 1% of TCE) after 309 days of operation (Figure 2a).

Table 2: Summary of EVO mass balance in the GE columns.

Column	TOC In (g)	TOC Out (g)	% Retained
Plume	6.34	1.85	70.82
DNAPL (1st Addition)	6.18	0.09	98.54
DNAPL (2nd Addition)	6.89	0.19	97.24

The SiREM column study consisted of two columns that both contained a TCE DNAPL source zone. The SiREM DNAPL phase columns were designed to estimate the extent of dissolution created by the dechlorination process. Following EVO amendment and bioaugmentation with KB-1® to the experimental column, TCE was dechlorinated to cDCE and concomitant decreases in sulphate were observed (Figure 2b). Monitoring sulphate is important because it is an indicator of reducing conditions required by the dechlorinating organisms. Sulphate also competes with dechlorinating bacteria for electron donor and imposes an electron donor demand which needs to be considered

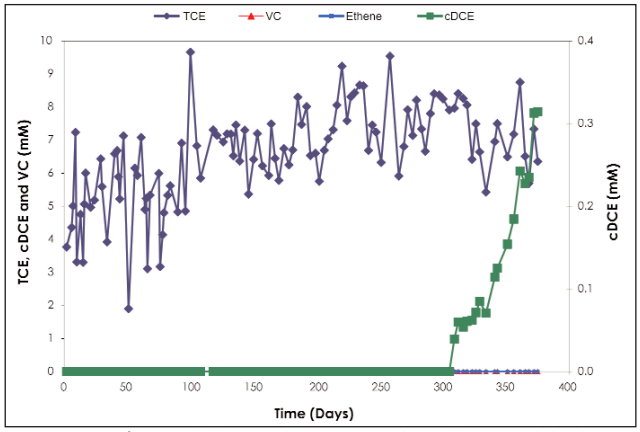

Figure 2a: Summary of VOC and ethene trends in GE DNAPL phase column amended with EVO.

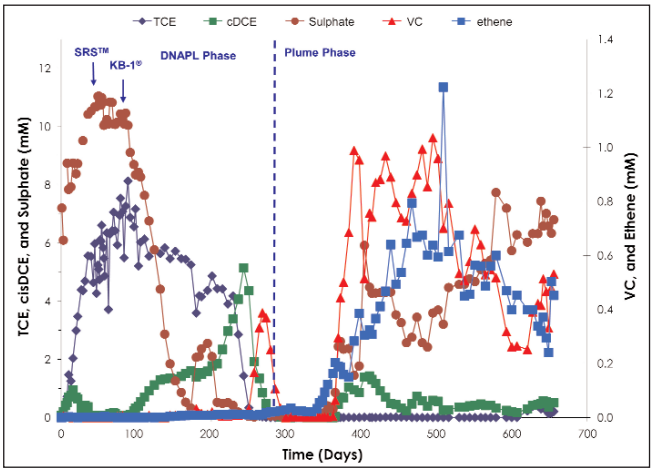

Figure 2b: Summary of VOC, ethene and sulphate trends in SiREM DNAPL phase and plume phase columns amended with EVO.

when designing remediation systems. TCE dechlorination was complete by day 257 with cDCE as the main chlorinated ethene measured in the effluent along with low levels of VC and ethene. Dechlorination of cDCE continued through VC to ethene by day 300. In contrast, the control column (which did not receive electron donor or culture) showed no evidence of TCE dechlorination (data not shown). One possibility for the differences in activity in the SiREM and GE bioaugmented DNAPL columns may be the different residual saturations of TCE in the columns (20% for the GE column relative to 10% for the SiREM column). DNAPL source zones are often heterogeneous environments, and, as a result, their observed activity will vary spatially.

During the most active phase of TCE biodegradation, the pH in the SiREM column effluent decreased to near 6, well below the optimum pH for reductive dechlorination of 6.8 to 7.5 (Middledorp et al., 1999). This suggests that the acid-buffering capacity of the site materials was not sufficient to maintain a neutral pH during acid production from dechlorination and donor fermentation. The addition of bicarbonate in the influent AGW ameliorated the pH decrease to near neutral values. Dhc and vcrA gene copies did not increase during the DNAPL phase of the SiREM column suggesting that other organisms (e.g. *Geobacter*) were responsible for the TCE to cDCE dechlorination. These organisms may have a higher tolerance to TCE, allowing them to thrive in the DNAPL zone (Amos et al., 2009).

Dissolution enhancement factors may be calculated as the total moles removed in a treatment column divided by the total moles removed due to TCE dissolution in a control column (Cope and Hughes, 2001). The amount of DNAPL added to each of the SiREM DNAPL columns was different (Table 1). Therefore, dissolution from the EVO and control columns were compared as a percentage of the total chlorinated mass added to each column. Figure 3 shows that, before biostimulation of the EVO column, the normalised dissolution in the control column was slightly greater.

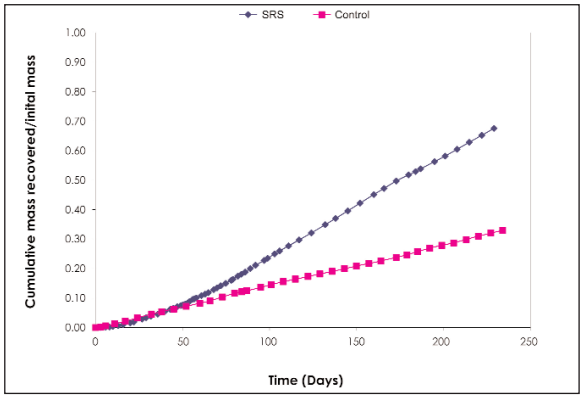

Figure 3: Enhanced dissolution in SiREM DNAPL phase column.

After biostimulation on day 47, the cumulative mass removal of TCE in the EVO column began to increase relative to the control column and continued to increase after bioaugmentation with KB-1® on day 78. By day 229 biostimulation and bioaugmentation had increased the rate of mass removal relative to the control column by a factor of 2.1.

4.2 Plume Phase Results

In contrast to the GE DNAPL column, the GE plume phase column showed complete dechlorination of 250 mg/L aqueous phase TCE to ethene within 250 days of operation (Figure 4). A subsequent breakthrough of cDCE and VC and decrease in ethene production was observed after 300 days and was likely due to depletion of the electron donor. Additional EVO was added to the column at day 371 and on day 380 the DNAPL phase and the plume phase columns were linked together such that the effluent of the DNAPL phase column became the influent for the plume phase column, increasing the concentration of TCE entering the plume phase column. After linkage of the columns, TCE was rapidly dechlorinated to ethene within the plume phase column although the main compound in the effluent of the linked columns was cDCE. After 40 days of operation as linked columns the effluent pH was observed to drop from 7.5 to below 6.0 and the production of ethene slowed. Similar to the SiREM DNAPL column, addition of bicarbonate resulted in an increase in pH and a resumption of previous ethene production (Figure 5). These results emphasise the importance of monitoring and controlling pH in actively dechlorinating bioremediation systems.

After the DNAPL was depleted in the SiREM column the influent AGW was amended with approximately 300 mg/L of TCE to simulate a TCE plume phase down gradient of a TCE DNAPL source zone. Effluent TCE concentrations remained below the

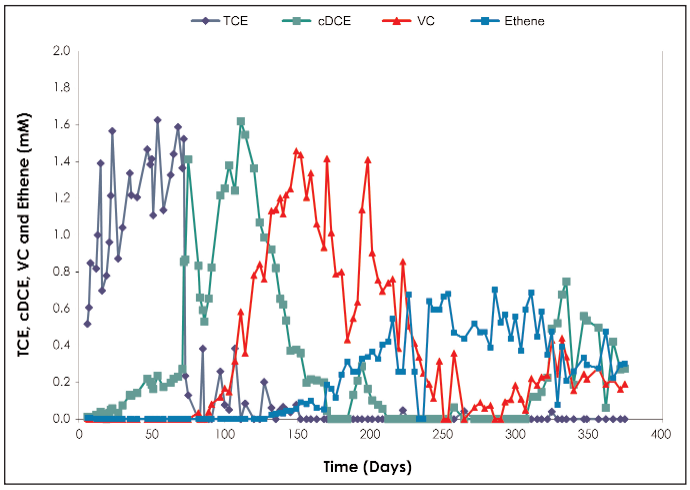

Figure 4: Summary of VOC and ethene concentrations in GE plume phase column (EVO amended, no DNAPL phase).

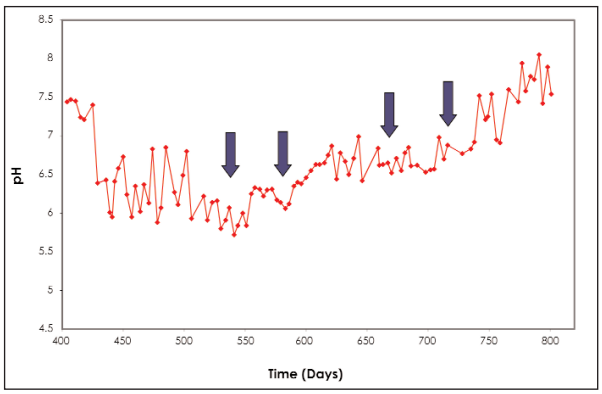

Figure 5: Summary of pH trends in GE column (arrows indicate bicarbonate additions).

detection limit over the plume phase of the study (Figure 2b). cDCE, VC, and ethene concentrations were detected throughout the plume phase indicating that complete dechlorination to ethene was possible. The effluent sulphate concentration remained low at the start of the plume phase until day 348, after which sulphate breakthrough was observed. Dechlorination to VC and ethene continued at sulphate concentrations of approximately 570 mg/L (approximately 50% of influent) indicating that dechlorinating organisms successfully competed with sulphate reducers for available electron donor. This observation is important because sulphate reduction typically precedes complete dechlorination, which increases the amount of electron donor required to achieve complete dechlorination. If complete dechlorination occurs prior to sulphate reduction savings in electron donor can be realised.

Following TCE decrease and the production of VC and ethene in the SiREM plume phase column Dhc and vcrA counts increased from near non-detect to 1 X 10^8 and 8 X 10^7 gene copies/L, respectively. The concentrations of Dhc measured here are consistent with levels where ethene production is expected (Lu et al., 2006). These results indicate that the dechlorinating organisms were tolerant of the stresses of high TCE concentrations and transient low pH conditions, allowing dechlorination of TCE to ethene once the DNAPL was depleted and the pH returned to near neutral.

4.3 DuPont Plume Zone (+Sulphate and Sulphate-Free Columns)

The DuPont columns differed from the GE and SiREM columns in that no DNAPL phase was present. These experiments tested the effect of sulphate on reductive dechlorination in columns simulating plume conditions with (+sulphate) and without sulphate in the influent AGW. Initially, EVO was added at the stoichiometric electron donor demand of each column and as a result the +sulphate column received four times that of the sulphate-free column. After EVO addition, sulphate was quickly depleted and TCE was dechlorinated to cDCE in both columns. However, VC and ethene were produced only in the +sulphate column (Figure 6). After 325 days of operation, evidence was obtained that the added EVO had been depleted (TCE breakthrough in the sulphate-free column, decreased VC production and sulphate breakthrough in the +sulphate column). EVO was re-amended on day 445. Due to a complication during the addition, the amount of EVO loaded onto each column is not certain. However, dechlorination in both columns responded rapidly to the added electron donor resulting in depletion of TCE and production of cDCE and VC. Evidence of electron donor limitation (sulphate breakthrough and/or decreased

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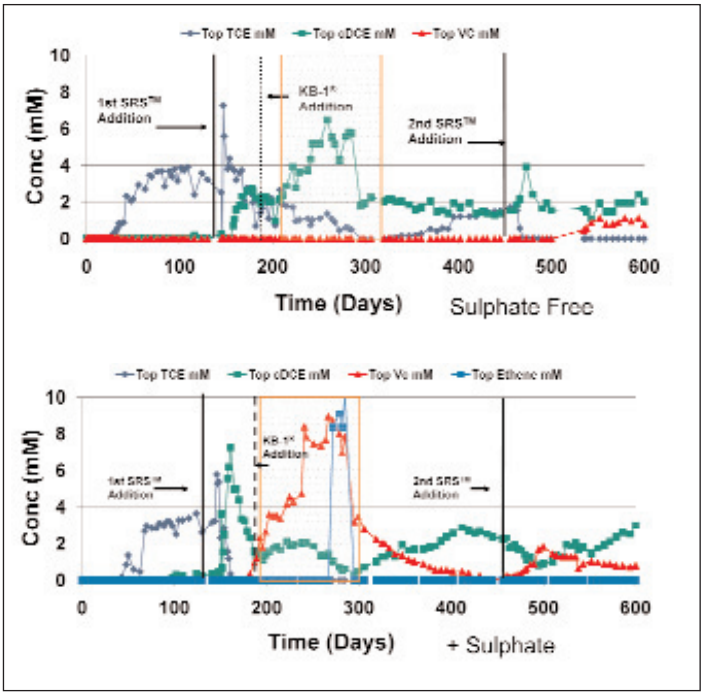

Figure 6: Summary of VOC and ethene concentrations in DuPont plume phase columns. Shaded areas indicates period during which data are only qualitative due to an analytical error.

production of VC) was observed again 65 days after the second addition of EVO. In both systems, TCE dechlorination to cDCE and VC was observed to occur rapidly within the first 40-50 cm of the column. Unlike the GE and SiREM columns, pH in these columns remained neutral and pH buffering was not required. These results suggest that given enough electron donor, the presence or absence of sulphate had no effect on dechlorination in this system.

5. CONCLUSIONS

Based on the results of these column studies the following conclusions are drawn.

- The EVO partitioned into the TCE DNAPL phase and supported reductive dechlorination of TCE to ethene in the presence of DNAPL (250 – 1000 mg/L TCE). These results indicate that microbial treatment is a viable remedial strategy for TCE DNAPL source zones.
- The application of a DNAPL partitioning electron donor had the beneficial effects of physically keeping the electron donor in the source zone and enhancing DNAPL dissolution by a factor of 2.1.
- Given sufficient electron donor and system activity, sulphate (present at elevated concentrations in the site groundwater) does not appear to inhibit the dechlorination of VC to ethene. Complete dechlorination of TCE can occur in the presence of sulphate.
- High rates of dechlorination and biotransformation of the EVO may result in acid production that can impact system pH and negatively affect rates of dechlorination. However, dechlorination associated pH drop can be mitigated by bicarbonate addition.
- Although enhanced dissolution occurs in the source zone, the plume zone is also important in achieving treatment objectives of complete dechlorination to ethene. When treating DNAPL source areas the electron donor requirements of the plume phase should also be considered.

Additional results from the SABRE studies including site characterisation methods, numerical modelling techniques and field data results will be presented in other CL:AIRE bulletins.

ACKNOWLEDGMENTS

The SABRE project team comprises (in no particular order): Acetate Products, Akzo Nobel, Archon Environmental, British Geological Survey, Chevron, DuPont, ESI, General Electric, Geosyntec Consultants, Golder Associates, Honeywell, Scientifics, Shell Global Solutions, Terra Systems, University of Edinburgh, University of Sheffield and CL:AIRE.

The SABRE project team would like to acknowledge the in-kind and direct financial contributions made by each participating organisation. SABRE is funded through the DTI Bioremediation LINK programme (http://www.clarrc.ed.ac.uk/link/) with specific financial contributions from BBSRC, DTI, the Environment Agency, EPSRC and NERC, which are gratefully acknowledged.

This bulletin was prepared by members of the Project SABRE Laboratory Work Package team, specifically: Erin Mack and JoAnn Payne (Dupont, Newark, DE, USA), Jeff Roberts and Sandra Dworatzek (SiREM, Guelph, Ontario Canada), Mark Harkness, Angela Fisher, (GE Global Research, Schenectady, NY, USA), Michael Lee (Terra Systems, Inc, Wilmington, DE, USA). Assistance in data interpretation and bulletin preparation was kindly provided by Carolyn Acheson (US Environmental Protection Agency, Cincinnati, OH, USA). We thank Perry McCarty (Stanford University) for his interest, valuable advice, and discussions throughout the project and the SABRE Modelling Work Package team for all of their hard work and useful input.

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