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# Evaluation of a laboratory-scale bioreactive *in situ* sediment cap for the treatment of organic contaminants

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ABSTRACT

The development of bioreactive sediment caps, in which microorganisms capable of contaminant transformation are placed within an *in situ* cap, provides a potential remedial design that can sustainably treat sediment and groundwater contaminants. The goal of this study was to evaluate the ability and limitations of a mixed, anaerobic dechlorinating consortium to treat chlorinated ethenes within a sand-based cap. Results of batch experiments demonstrate that a tetrachloroethene (PCE)-to-ethene mixed consortium was able to completely dechlorinate dissolved-phase PCE to ethene when supplied only with sediment porewater obtained from a sediment column. To simulate a bioreactive cap, laboratory-scale sand columns inoculated with the mixed culture were placed in series with an upflow sediment column and directly supplied sediment effluent and dissolved-phase chlorinated ethenes. The mixed consortium was not able to sustain dechlorination activity at a retention time of 0.5 days without delivery of amendments to the sediment effluent, evidenced by the loss of *cis*-1,2-dichloroethene (*cis*-DCE) dechlorination to vinyl chloride. When soluble electron donor was supplied to the sediment effluent, complete dechlorination of *cis*-DCE to ethene was observed at retention times of 0.5 days, suggesting that sediment effluent lacked sufficient electron donor to maintain active dechlorination within the sediment cap. Introduction of elevated contaminant concentrations also limited biotransformation performance of the dechlorinating consortium within the cap. These findings indicate that *in situ* bioreactive capping can be a feasible remedial approach, provided that residence times are adequate and that appropriate levels of electron donor and contaminant exist within the cap.



## 1. Introduction

The management and remediation of contaminated aquatic sediments pose major technical and economic challenges. Treatment of contaminated sediment sites with *in situ* caps has become an established practice that can provide advantages over alternative methods in certain settings (Reible et al., 2003). Clean sand has traditionally been employed as capping material, and remains a large component of many field-scale capping applications. Sand-based caps have the potential to delay contaminant breakthrough when diffusive transport dominates (Go et al., 2009; Thoma et al., 1993), but eventual contaminant breakthrough remains a source of concern. Additionally, traditional sand

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caps are less effective at sites where groundwater seepage or mobile contaminants (i.e., low $K_{oc}$) are present (Go et al., 2009). Research studies have focused on *in situ* sequestration (Cho et al., 2007; Zimmerman et al., 2004), *in situ* transformation (Krumins et al., 2009; Lowry and Johnson, 2004), and the development of active caps which incorporate reactive and/or sorptive constituents designed to reduce contaminant and bioavailability (Choi et al., 2009; Hyun et al., 2006; Jacobs and Förstner, 1999; McDonough et al., 2007; Murphy et al., 2006; Reible et al., 2007). Ideally, active caps eliminate the risk of contaminant breakthrough into the overlying water column, and can potentially be implemented at sediment sites with groundwater seeps and relatively mobile contaminants.

The employment of physicochemical-based active caps appears promising, but possible limitations (e.g., high material costs, sorption and reaction capacities) have stimulated the consideration of *in situ* bioreactive caps, in which contaminant biotransformations are designed to occur within the cap matrix to produce environmentally-acceptable reaction products. Enhanced *in situ* bioremediation, through biostimulation and bioaugmentation, has proven to be a successful groundwater remediation technology for a diverse range of contaminants (Löffler and Edwards, 2006). Adaptation of these principles to subaqueous sediment remediation has not been demonstrated, prompting the recent identification of *in situ* bioremediation as a priority research and development need (SERDP/ESTCP, 2008). Biologically-based active caps have the potential to maintain reactivity over long periods of time and could serve as a sustainable remedial option if microorganisms capable of biotransformation are present and necessary metabolic requirements are met.

Previous studies that investigated the activity of microbial populations within a sediment cap demonstrated that microorganisms indigenous to underlying sediment, including organisms capable of contaminant biotransformation, are able to colonize the overlying cap and possibly participate in contaminant bioattenuation processes (Himmelheber et al., 2009). Bioaugmentation of microorganisms within a cap, as opposed to intrinsic colonization (defined here as the natural redistribution of microorganisms native to the sediment into the cap matrix), could provide enhanced degradation capacity and minimize the potential for contaminant release to benthic and aqueous receptors. Such a bioaugmentation strategy was recently evaluated by the US Geological Survey (USGS) as a means to reductively dechlorinate a mixture of chlorinated ethenes, ethanes, and methanes present in a groundwater seep discharging into a tidal wetland (Majcher et al., 2007). A mixed, anaerobic culture was enriched from the site (Lorah et al., 2008) and incorporated into an organic-based matrix that was placed at the sediment-water interface. This bioreactive mat successfully treated the chlorinated contaminants prior to discharge (Majcher et al., 2009). Although the bioreactive mat was constructed on the banks of a tidal wetland (i.e., not completely subaqueous) and the design is not immediately suitable for submergence (e.g., buoyancy restrictions, delivery of bioaugmentation culture), the success of the approach supports the concept of bioreactive capping as an *in situ* remedial technique.

The USGS bioreactive mat was designed in part because the chlorinated organics present in the groundwater were undergoing only partial dechlorination in the sediment prior to discharge, a phenomenon commonly reported at sediment sites (Abe et al., 2009; Conant et al., 2004; Hamonts et al., 2009; Himmelheber et al., 2007; Lendvay et al., 1998; Lorah and Voytek, 2004; Majcher et al., 2007). Additionally, recent studies have demonstrated that anaerobic conditions develop within sediment caps subject to diffusive and upflow conditions (i.e., groundwater seeps) (Himmelheber et al., 2008, 2009). It is therefore expected that contaminated groundwater seeps will carry partially-degraded contaminants into the overlying anaerobic cap, thereby providing an opportunity for treatment by reductive biotransformations.

Detailed assessment of bioreactive *in situ* sediment caps has not been previously undertaken and little is currently known about the feasibility of bioreactive caps, particularly their limitations and maintenance requirements. The objective of this work was to establish an actively dechlorinating microbial consortium within a simulated overlying cap and to determine how contaminant mass flux and electron donor amendments influenced bioreactive cap performance. More specifically, the bioreactive cap experiments were designed to determine whether or not amendments are necessary to sustain complete reductive dechlorination by an active microbial community. Chlorinated ethenes were utilized as the contaminants due to their frequent occurrence as groundwater contaminants, their presence in groundwater seeps, and their greater mobility relative to other sediment contaminants (e.g., chlorinated benzenes, polychlorinated biphenyls). Batch reactor and bioaugmented column studies were conducted to assess bioreactive cap performance over a range of electron donor and contaminant conditions.

## 2. Materials and methods

### 2.1. Chemicals

PCE (99+%, Sigma–Aldrich, St. Louis, MO), TCE (99.5%, Sigma–Aldrich), *cis*-DCE (97%, Acros Organics, Morris Plains, NJ), *trans*-DCE (99.7%, Acros Organics), and 1,1-DCE (99.9%, Acros Organics) were obtained in neat liquid form. Vinyl chloride (8%/$N_2$ balance), ethene (99.5%), ethane (99.5%), and methane (99%) were obtained from Matheson Tri-Gas (Parsippany, NJ). Sodium bicarbonate, potassium chloride, magnesium chloride, and calcium chloride were used in the preparation of simulated groundwater and were purchased from Fisher Scientific (Pittsburgh, PA). Sodium lactate syrup (60% vol/vol, Fisher Scientific) was used during the preparation of stock lactate solutions. Potassium bromide, calcium sulfate, and potassium phosphate were purchased from Fisher Scientific and used for IC standards and non-reactive tracer studies.

### 2.2. Batch reactors

Batch reactors were established in triplicate and consisted of Anacostia River (Washington, D.C., USA) sediment porewater, dissolved-phase PCE, and a mixed PCE-to-ethene dechlorinating consortium. A PCE-to-ethene dechlorinating mixed

consortia referred to as OW served as the inoculum. The OW consortia, which is capable of complete reductive dechlorination of PCE to ethene, has been described previously (Daprato et al., 2006). The OW culture has been found to contain multiple dechlorinating microorganisms, including *Dehalococcoides* species, and known reductive dehalogenases including *tceA*, *vcrA*, and *bvcA* (Daprato et al., 2006).

Three 25 mL aliquots of OW culture were transferred to 70 mL serum bottles pre-capped with Teflon®-faced butyl septa and sparged with $N_2$ gas for 15 min to remove oxygen from the empty bottles. The collected OW aliquots were then sparged with $N_2$ gas for 15 min in attempt to remove residual chlorinated ethenes, methanol, and volatile fatty acids from the batch reactors. Sediment effluent was collected from a sediment column that was supplied only with simulated groundwater and dissolved-phase PCE (Himmelheber et al., 2007). The composition of simulated groundwater was slightly modified from that described by Dries et al. (2004) and consisted of 3.5 mM $NaHCO_3$, 0.1 mM KCl, 0.25 mM $MgCl_2$, 0.75 mM $CaCl_2$, and resazurin as a redox indicator. Sediment effluent was collected under anoxic conditions and 25 mL of effluent were added directly to batch reactors containing the OW consortium. Dissolved-phase PCE was obtained from a saturated stock solution containing neat PCE in contact with anaerobic, sterilized simulated groundwater. Stock PCE concentrations were quantified immediately prior to injection into the batch reactors. Approximately 16 μmol of dissolved-phase PCE was added to each microcosm using a 10 μL Hamilton glass syringe. All reactors were wrapped in foil and incubated at 20° C on an orbital shaker operated at 150 rpm. Chlorinated ethenes, ethene, ethane, and methane concentrations were determined from headspace samples of the microcosms.

### 2.3. Bioreactive cap operation

Two one-dimensional (1-D) columns (designated herein as Bioreactive Cap A and Bioreactive Cap B) were constructed using 2.5 cm inside diameter (I.D.) glass chromatography columns × 30 cm in length (Spectrum Chromatography, Houston, TX) and equipped with custom-built stainless steel end plates (Dutton & Hall, Atlanta, GA). A 2.5 cm diameter disc of 80 mesh stainless steel (Small Parts, Inc., Miami Lakes, FL) was placed on the column end plates to retain sand grains within the column. A fabricated glass reservoir (15 mL) fitted with a stopcock was placed at the column effluent to allow for aqueous effluent sampling. The columns were packed with ASTM C-33 grade concrete sand (U.S. Silica, Mauricetown, NJ). This particular sand was selected because it is representative of the solids used for submerged sediment caps and was utilized in the Anacostia River Capping Demonstration Project (Reible, 2005). An elemental analysis of the sand was performed at the University of Georgia Laboratory for Environmental Analysis (see Supplementary Information, Table S.1). The dry, autoclaved sand was packed into the bioreactive columns under aerobic conditions in 5-cm increments with vibration along the outside wall of the column. Three pore volumes of $N_2$-sparged, autoclaved simulated groundwater were flushed through the columns to check for leakage and to ensure anaerobic conditions. The columns were then inoculated by flushing the columns with three pore volumes of the OW culture suspension.

Following inoculation, the two sand cap columns were wrapped in foil to avoid exposure to light then connected in series with an upflow column packed with Anacostia River sediment as depicted in Fig. 1. The sediment column effluent, which was provided only with simulated groundwater and dissolved-phase PCE, served as the influent for the bioreactive sand columns. Therefore, the influent for the sand columns consisted of sediment effluent and a mixture of partial PCE-dechlorination products, similar to the conditions that would be anticipated in a submerged sediment capping scenario subject to a PCE-contaminated groundwater seep. Table 1 provides a summary of experimental conditions employed for each bioreactive sand column. Chlorinated ethene and ethene concentrations in the effluent of Bioreactive Caps A and B were normalized on a molar basis to total chlorinated ethenes and ethene eluted per sample to reduce scatter in concentration data and to monitor product distribution.

#### 2.3.1. Bioreactive Cap A

Bioreactive Cap A was designed to assess the ability of sediment effluent to maintain an external dechlorinating community in a cap, simulating a bioreactive cap inoculated with a mixed dechlorinating consortia and operating under reducing conditions. Prior to inoculating Bioreactive Cap A, a tracer test was conducted with a pulse injection of 100 mg $L^{-1}$ (1.25 mM) bromide obtained from an autoclaved, sparged stock solution of potassium bromide in simulated groundwater. A total of 1.2 pore volumes were flushed through the column, collected with a fraction collector, and analyzed via ion chromatography. Three pore volumes of simulated groundwater were then flushed through the column following the tracer test to remove residual bromide prior to inoculation. A 200 mL aliquot of aqueous OW culture was obtained for inoculation and stored in a 160 mL serum bottle that had previously been capped with a Teflon®-faced butyl septum and sparged with $N_2$ for 15 min to remove oxygen. The 200 mL aliquot was tested for its dechlorination ability in batch conditions by spiking with PCE and methanol. After successfully dechlorinating PCE to ethene (Supplementary Information, Fig. S.1A), 1.5 pore volumes of the OW culture were supplied to the column at a flow rate of 2.2 mL $h^{-1}$ (1-day residence time). Following a 24-h attachment period during which there was no flow, Bioreactive Cap A was connected in series with the sediment column from 67 to 83 sediment pore volumes. The unamended sediment column effluent served as the influent for the duration of the Bioreactive Cap A experiment.

#### 2.3.2. Bioreactive Cap B

The Bioreactive Cap B experiment was designed to simulate a dechlorinating bioreactive cap operating under reducing conditions, but differed from Cap A in that the influent for this experiment was supplied at various flow rates and periodically spiked with amendments. Thus, Bioreactive Cap B demonstrates the impact of contaminant influx and the presence of reducing equivalents on the capacity of sediment column effluent to maintain an external dechlorinating

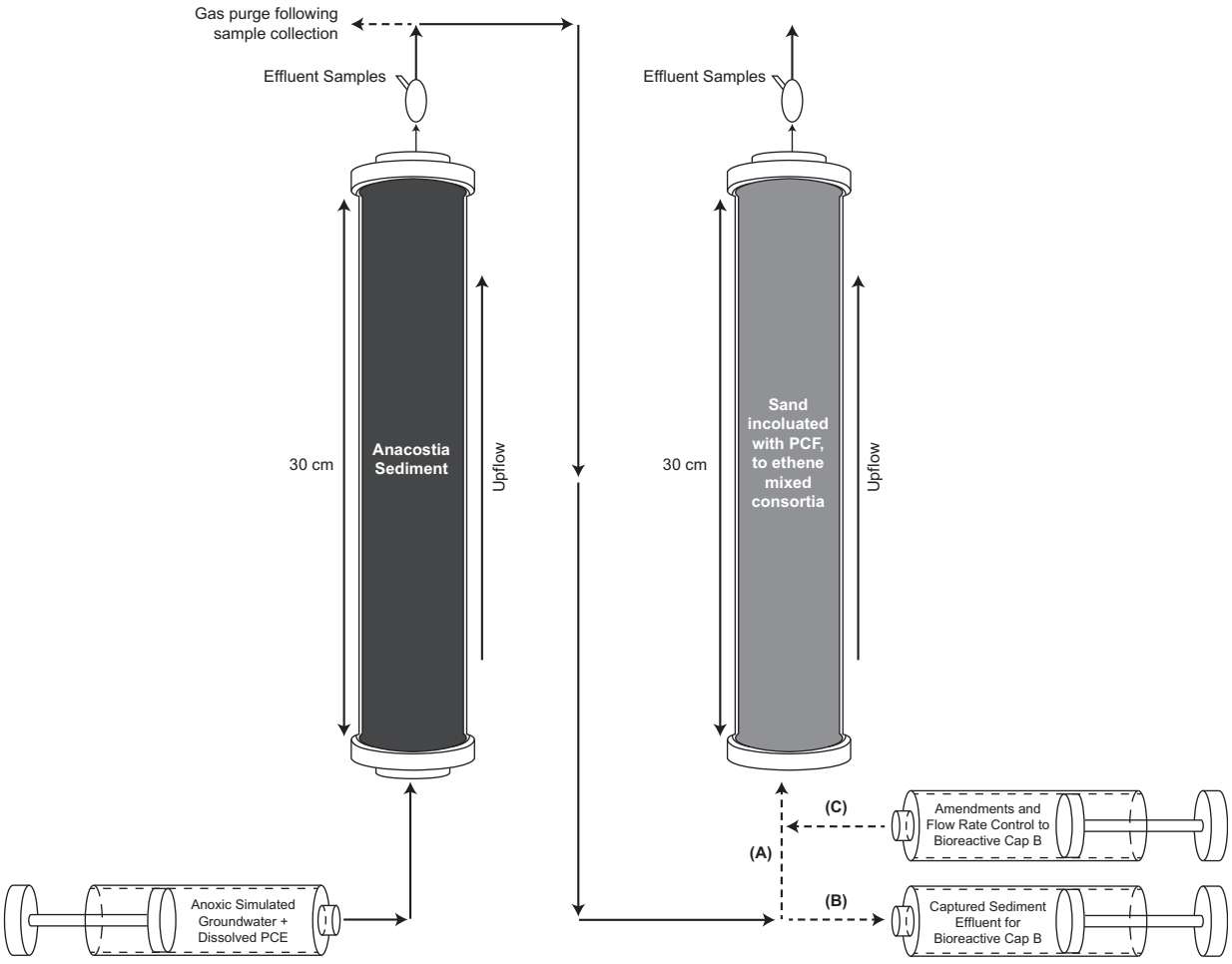


**Fig. 1 – Conceptual schematic of laboratory simulation of a bioreactive sand cap placed in series with an anaerobic sediment bed subject to a PCE-contaminated groundwater seep. (A) Sediment effluent was directly supplied to Bioreactive Cap A. (B) Sediment effluent was initially captured via a syringe for Bioreactive Cap B, spiked with amendments, then (C) supplied to Bioreactive Cap B at select flow rates.**

community. An aliquot of OW culture was retrieved and sparged with nitrogen prior to inoculation as described for Bioreactive Cap A. The aliquot of OW culture again demonstrated the ability to completely dechlorinate PCE to ethene in batch culture (Supplementary Information, Fig. S.1B). A total of 1.7 pore volumes of OW culture was then supplied to the column at a flow rate of 2.6 mL $h^{-1}$ (1-day residence time), followed by a no-flow attachment period of one day. Unlike Bioreactive Cap A, Bioreactive Cap B was not immediately connected to the sediment column effluent, but rather positive-control experiments were conducted to ensure the inoculated column could completely dechlorinate *cis*-DCE to ethene when provided DCB-1 media, Wolin vitamins, and 5 mM lactate as an electron donor and carbon source. Following this demonstration of complete dechlorination in the cap under optimal conditions (Supplementary Information, Fig. S.2), one pore volume of anaerobic simulated groundwater was flushed through the column to remove these constituents from the system prior to the introduction of sediment effluent. Sediment column effluent was obtained from 146 to 180 sediment pore volumes to serve as Bioreactive Cap B influent.

For Bioreactive Cap B, sediment column effluent was captured under anoxic conditions by connecting an empty, gas-tight syringe to sediment effluent tubing and allowing the aqueous flow to gradually fill the syringe at the same rate of sediment column influent (5.5 mL $h^{-1}$). Once the effluent syringe had been filled, it was immediately transferred to a separate syringe pump and introduced into the sand column as the influent. This method allowed for manipulation of flow rates within the sand column and for addition of electron donor and acceptor to the influent prior to connection with the sand column. The electron donor used for this study was lactate, which was obtained from a 100 mM stock solution in autoclaved, sparged simulated groundwater. Lactate was supplied to the bioreactive sand column (Cap B) at a concentration of 5 mM from 0 to 13.3 pore volumes (Table 1).

The experimental conditions employed for Bioreactive Cap B were designed to gradually decrease aqueous residence times, as well as electron donor concentrations, to determine

**Table 1 – Summary of experimental conditions for sand column experiments.**

| Parameter | Bioreactive Cap A | Bioreactive Cap B |
|---|---|---|
| Pore volume (PV)[a] (mL) | 62.72 | 61.82 |
| Porosity ($n$)[a] ($cm^3$ void ($cm^3$ total)$^{-1}$) | 0.41 | 0.41 |
| Connected in series to sediment column (sediment pore volumes) | 67.0–83.2 | 146.0–180.0 |
| Experimental flow rate ($Q$) (mL $h^{-1}$) | 5.46 | 1.29; 2.58; 5.46 |
| Porewater velocity ($v$) (cm $day^{-1}$) (Darcy velocity (cm $day^{-1}$)) | 62.67 (25.88) | 14.99; 29.98; 63.59 (6.10); (12.20); (25.88) |
| Peclet number ($Pe$)[b] (dimensionless) | 80.5 | N/A[c] |
| Alterations to influent | None | Addition of Lactate<br>Addition of *cis*-DCE<br>Decrease of flow rate |
| Influent chloroethene concentration (μM total chlorinated ethenes) | 16.19 ± 11.06[d] | 0–3.44 PV: 200 ± 42[d]<br>3.44 PV to end: 34 ± 3.6[d] |

a Estimated from mass difference between dry and wet packed columns.
b Obtained with the CFITM3 breakthrough curve fitting program under equilibrium constraints.
c Tracer test not performed.
d Average ± one standard deviation.

limitations on dechlorination (Table 1). The influent flow rate for Bioreactive Cap B was increased step-wise from 1.3 mL $h^{-1}$ (2-day retention time), to 2.6 mL $h^{-1}$ (1-day retention time) to 5.5 mL $h^{-1}$ (0.47-day retention time). From 0 to 3.4 sand pore volumes, additional *cis*-DCE was provided to the influent to ensure chlorinated ethenes were present due to complete dechlorination of PCE to ethene in the sediment column effluent prior to connecting Bioreactive Cap B. *cis*-DCE was chosen assuming partial, intrinsic PCE dechlorination would occur in sediment beds, based on prior research findings (Himmelheber et al., 2007). The *cis*-DCE was obtained from a saturated stock solution of *cis*-DCE (i.e., NAPL present) in autoclaved, sparged simulated groundwater and supplied to the influent at a concentration of 200 ± 42 μM. After 3.4 pore volumes, however, the only source of chlorinated ethenes to Bioreactive Cap B was the sediment effluent. Lactate (5 mM) was provided from 0 to 13.3 pore volumes, at which point it was removed from the influent and no electron donor was provided for the remainder of the experiment.

### 2.4. *Analytical methods*

PCE, TCE, DCE isomers, VC, ethene, ethane, and methane concentrations were determined from the headspace of 5 mL aqueous effluent samples, which were analyzed using an Agilent 6890 gas chromatograph (GC) equipped with a flame ionization detector (FID), as described previously (Carr and Hughes, 1998). Bromide was measured using a Dionex DX-100 ion chromatograph with a Dionex AG4A IonPac guard column and Dionex AS4A IonPac column at a flow rate of 1.5 mL/min and an ED40 electrochemical detector.

## 3. Results and discussion

### 3.1. *Batch reactors*

The OW culture successfully dechlorinated PCE to ethene when provided only sediment effluent and dissolved-phase PCE (Fig. 2). Complete PCE dechlorination to ethene was achieved after 19 days of incubation. Chlorinated ethene mass balance was within 10% for each time point except day 12, when chloroethene mole totals were 124% of the initial dissolved-phase PCE introduced to the batch reactors. This discrepancy arose because one of the triplicate reactors recorded unusually high concentrations of VC, despite balanced ethene concentrations at the end of the experiment. This is reflected in the relatively high standard deviation of VC at day 12. Duplicate analysis at the same time point yielded similar results. Regardless of this isolated analytical discrepancy, the presence of VC and ethene indicates that dechlorinating species within the OW consortium, specifically Dehalococcoides, remained active for at least one dechlorination cycle when provided only sediment effluent and a dissolved-phase electron acceptor (PCE). Thus, the sources of carbon, electron donor, and micronutrients were provided by the sediment effluent or from microbial biomass (Adamson and Newell, 2009). Methane concentrations rose steadily during the dechlorination of PCE (Fig. 2), indicating that methanogenic populations were also able to remain active when provided only sediment effluent. These data suggest that dechlorinating species within a bioreactive cap inoculated with a methanogenic mixed consortia may have to

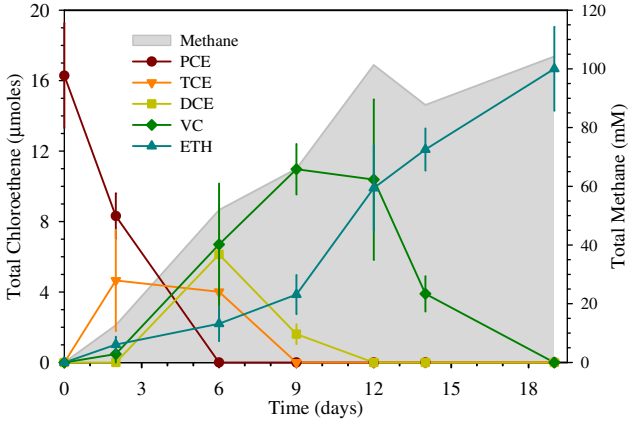


**Fig. 2 – Batch microcosm results of OW culture provided only PCE and sediment effluent. Chlorinated ethenes are reported as the sum of aqueous and gas phases within the microcosms. Error bars represent one standard deviation calculated from triplicate reactors. The shaded background area corresponds to methane production (mM) at each time point and is referenced to the right vertical axis. Total methane is calculated as the sum of aqueous and gas phase methane.**

compete with methanogens for electron donors, which could result in reduced dechlorination efficiency over time. Methanogens and other microbial populations indigenous to the sediment are also expected to populate the cap material (Himmelheber et al., 2009) and may, therefore, compete for electron donor and other nutrients.

### 3.2. *Bioreactive Cap A*

The pore volume of Bioreactive Cap A was estimated to be 62.7 mL from differences between wet and dry column mass and assuming complete water saturation (Table 1). The non-reactive tracer test conducted at the onset of Bioreactive Cap A operation yielded a symmetrical breakthrough curve, indicative of the absence of immobile regions of water (see Supplementary Information, Fig. 2). The measured tracer BTC, expressed as the relative concentration versus number of dimensionless pore volumes applied, was fit to an analytical solution of the one-dimensional advective-dispersive reactive (ADR) transport equation using the CXTFIT model (van Genuchten, 1981). As anticipated, the fitted retardation factor ($R_F$) obtained from the tracer BTC was approximately equal to 1.0, indicating no detectable interactions between the solid phase and tracer during transport through the sand column. The fitted Peclet number (*Pe*) was approximately 81, yielding a hydrodynamic dispersion coefficient ($D_H$) of $2.8 \times 10^{-8}$ $m^2$ $s^{-1}$ and a hydrodynamic dispersivity ($\alpha_D$) of 0.37 cm. These data are consistent with values reported for similar water-saturated columns packed with graded sands, and indicate that advective flow and transport through the column was normal and not subject to physical non-equilibrium.

Chlorinated ethene effluent product distributions, normalized to moles of chlorinated ethenes and ethene eluted, are shown for Bioreactive Cap A (Fig. 3B). The applied influent flow rate of 5.5 mL $h^{-1}$ corresponded to a column residence time of 0.47 days. When Bioreactive Cap A was connected in series to the sediment column, *cis*-DCE was the predominant chlorinated ethene present in influent solution. The bioreactive sand column was initially able to dechlorinate *cis*-DCE to VC, but ethene was not detected (Fig. 3B). This dechlorination activity disappeared prior to 5 pore volumes, and eventually only 5% of the *cis*-DCE was dechlorinated to VC, indicating that Dehalococcoides activity was impaired. Methane data collected during the Bioreactive Cap A experiment reveal that microbes other than dechlorinators also lost activity, suggesting microbial impairment in the system as a whole and not just for the dechlorinating population (Fig. 3C). Based on data presented in Fig. 3B, the sediment column effluent was not able to sustain the dechlorinating consortium OW without additional amendments. Data were not collected to determine if non-contaminant stressors (e.g., ammonia) were present in the sediment, which could suppress microbial activity. However, previous research (Himmelheber et al., 2007) has demonstrated that microorganisms, specifically Dehalococcoides strains, can be stimulated in the Anacostia sediment with the addition of electron donor, suggesting that non-contaminant stressors were not a major concern in the system. Previous research (Himmelheber et al., 2007) has also demonstrated that microbial activity in the sediment column was limited by electron donor availability. It was therefore hypothesized that the levels of electron donor eluting from the sediment column effluent prevented the dechlorinating community in the sand cap from maintaining sufficient activity to achieve complete reductive dechlorination of the *cis*-DCE introduced to the sand cap column. A second possibility is that the relatively high flow rates through the sand cap column did not provide sufficient contact time between the contaminants and the dechlorinating community to achieve complete reductive dechlorination.

### 3.3. *Bioreactive Cap B*

To address the hypotheses raised above, the second sand column, Bioreactive Cap B, was operated at three different flow rates with and without the addition of lactate as an electron donor and *cis*-DCE as an electron acceptor (Table 1). The experimental conditions associated with Bioreactive Cap B are presented in Fig. 4A, while normalized chloroethene product distributions are shown in Fig. 4B. The pore volume for Bioreactive Cap B was estimated to be 61.8 mL from differences between wet and dry column mass and assuming complete water saturation (Table 1). Prior to supplying Bioreactive Cap B with sediment effluent, the inoculated column was able to completely dechlorinate *cis*-DCE to ethene when provided electron donor, carbon sources, vitamins, and reduced media; confirming the ability of the OW culture to achieve complete dechlorination within the column (Supplementary Information, Fig. S.2). After applying one pore volume of simulated groundwater, sediment effluent was supplied to Bioreactive Cap B, indicated as pore volume 0 in Fig. 4A and B. The influent for Bioreactive Cap B was the sediment column effluent from 146 to 180 sediment pore volumes, which contained a mixture of *cis*-DCE, VC, and ethene. The influent solution provided to Bioreactive Cap B was initially augmented with *cis*-DCE to yield a total influent chloroethene concentration of approximately 200 μM and 5 mM lactate, operated at a residence time of 2 days (flow rate = 1.29 mL $h^{-1}$) (Table 1). Incomplete dechlorination was observed during this period, with a mix of VC and ethene in the sand column effluent. From 3.4 to 5.7 pore volumes, only lactate was provided to the influent porewater (i.e., no *cis*-DCE was added) and the sediment effluent served as the sole source of chlorinated ethenes (ca. 34 μM). The sand column successfully achieved complete reductive dechlorination of the applied chlorinated ethenes to ethene during this period, demonstrating that with lactate addition and a residence time of 2 days the sand cap could detoxify the flux of chlorinated ethenes exiting the sediment column. These data, coupled with the lack of complete dechlorination during the previous condition (0–3.4 sand pore volumes) when additional *cis*-DCE was provided to the influent, suggests that high chloroethene concentrations entering the sand column limited the extent of dechlorination.

The results obtained from Bioreactive Cap B indicate that electron donor concentrations and contaminant residence times within the cap can impact dechlorination activity. Complete dechlorination was observed between 8.0 and 13.3 pore volumes when lactate was provided to the sand column

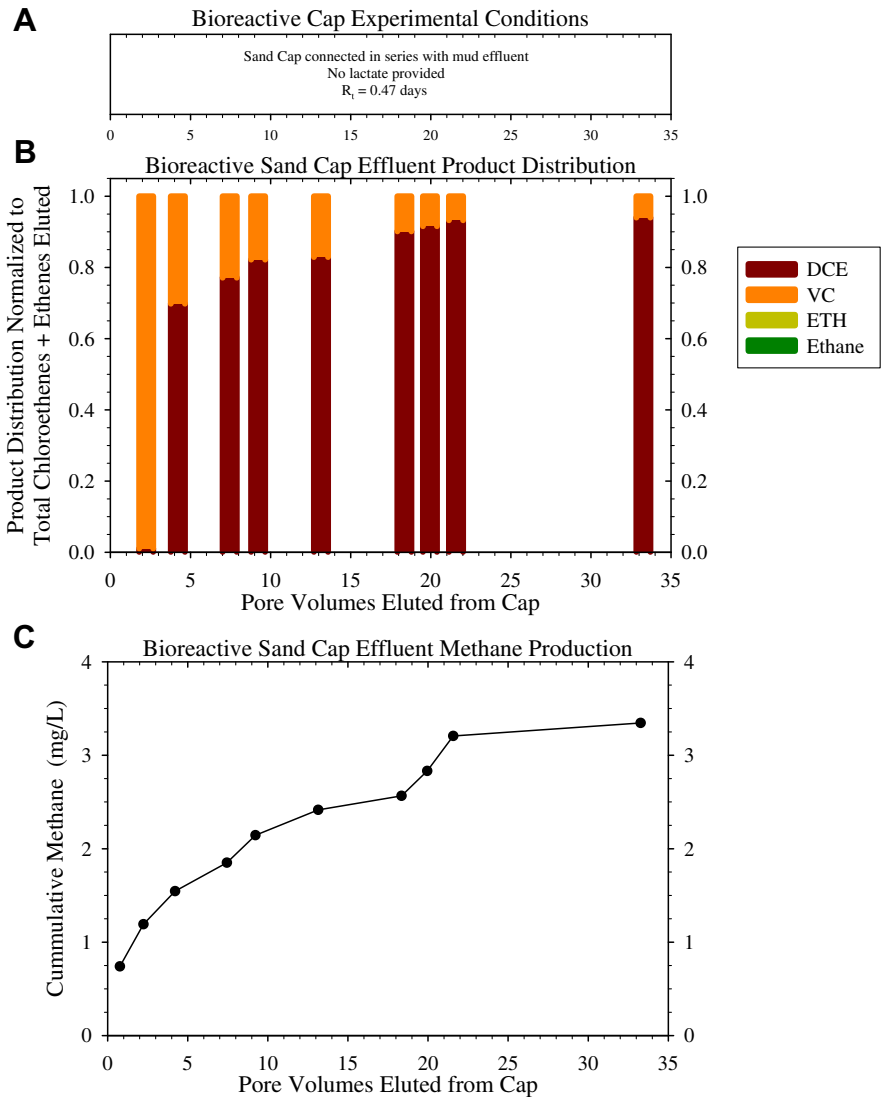


**Fig. 3 – A–B. (A) Operating conditions for Bioreactive Cap A. The effluent of the sediment column served as the influent of the sand column and was not amended with exogenous electron donors, electron acceptors, carbon sources, minerals, nor vitamins. (B) Effluent product distribution of Bioreactive Cap A inoculated with a PCE-to-ethene dechlorinating mixed consortia and connected in series with sediment column effluent between 68 and 83 sediment pore volumes. (C) Cumulative aqueous methane concentration in samples collected from Bioreactive Cap A effluent.**

despite relatively fast flow rates (0.47 residence time). When lactate was removed from the influent at 13.3 pore volumes, however, a mixture of chlorinated ethenes was observed in the effluent, indicating the importance of exogenous reducing equivalents to the sand column. Delivery of external electron donor is a common technique used to stimulate and enhance reductive dechlorination in groundwater aquifers (Anderson et al., 2003; Haas and Trego, 2001; Lendvay et al., 2003; Scow and Hicks, 2005) and may be necessary for bioreactive caps employing anaerobic biotransformations. Contaminant mass entering the cap also dictated performance, as noted above, since incomplete dechlorination was observed when additional *cis*-DCE was supplemented into the influent (0–3.4 PV) while complete dechlorination was observed when the cap was only treating sediment effluent (5–10 PV).

### 3.4. *Implications for capping*

The combined results from the batch study and the two sand columns suggest that the sediment effluent alone could not sustain complete dechlorination in a bioreactive cap over the range of residence times (0.5–2 days) examined in this study. Batch results showed complete dechlorination occurred after 19 days when the OW consortium was provided only sediment effluent, much longer than the 2 day retention times utilized for these column studies. However, at sites where diffusive conditions exist, or where groundwater seepage rates are significantly slower than those employed here, complete dechlorination could be achieved. For instance, at the USGS biomat pilot test described by Majcher et al. (2009), a bioreactive layer successfully dechlorinated a range of

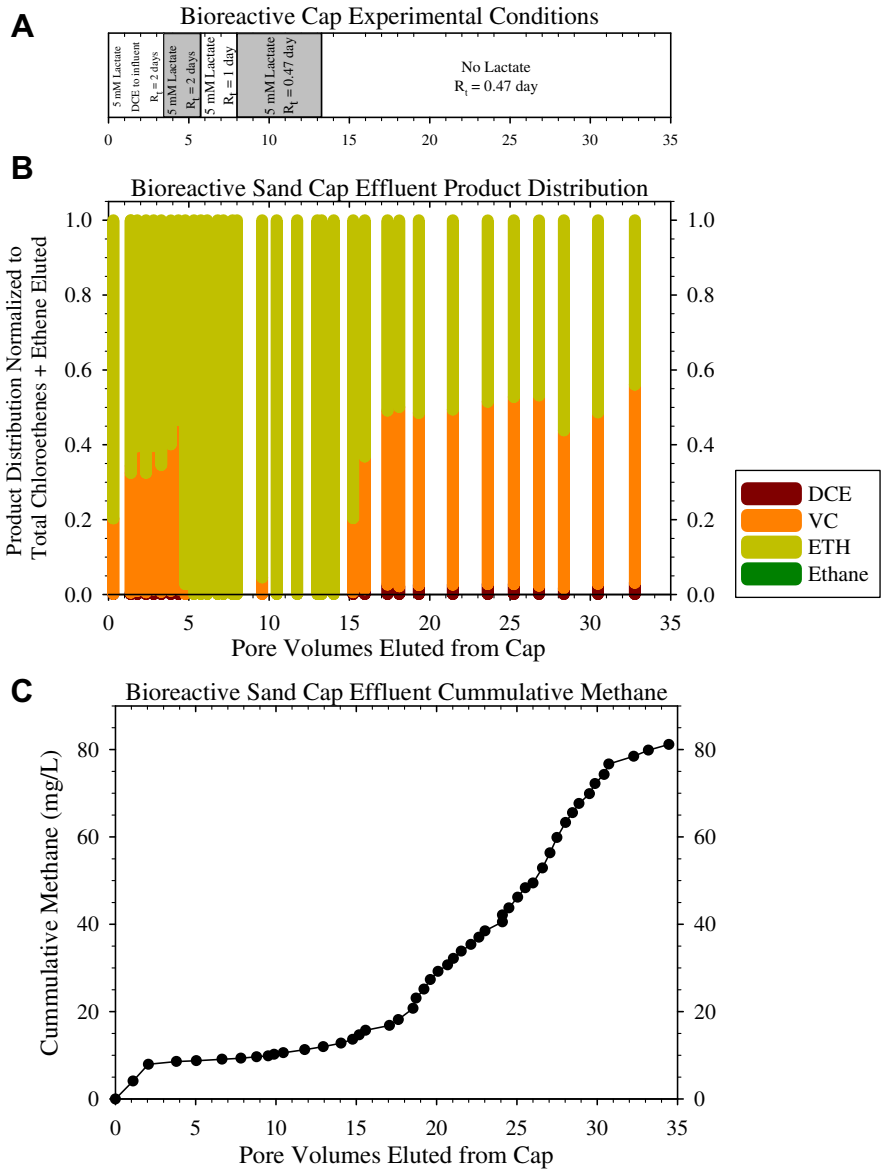


**Fig. 4 – A–B. (A) Operating conditions for Bioreactive Cap B. The effluent of the sediment column served as the influent of the Bioreactive Cap and was not amended with minerals nor vitamins. Exogenous electron donor (lactate), carbon sources (lactate), and electron acceptor (*cis*-DCE) were added where indicated. (B) Effluent product distribution of Bioreactive Cap B inoculated with a PCE-to-ethene mixed dechlorinating consortia and connected in series with sediment column effluent between 146 and 185 sediment pore volumes. (C) Cumulative aqueous methane concentration in samples collected from Bioreactive Cap B effluent.**

chlorinated aliphatics at a site where average hydraulic residence times in the reactive mat were assumed to be 8–14 days. Thissystem also included an organic layer composed of a mixture of peat, compost, and chitin to provide long-term electron donor.

## 4. Conclusions

Based on the results presented herein,

- Engineered controls may be needed to maintain microbial dechlorination activity, reduce contaminant flux, or increase contaminant residence time for bioreactive caps to achieve complete reductive dechlorination of dissolved chlorinated ethenes to ethene.
- Incorporation of electron donor was required to stimulate and sustain long-term contaminant biotransformations in a bioreactive cap under the conditions tested. At sites with lower seepage velocities, allowing for greater residence time in the cap, complete dechlorination without electron donor may be possible.
- The need for electron donor delivery in bioactive design could support greater cell numbers of degrading populations, resulting in greater degradation rates and possible deployment at sites with reasonably high contaminant flux (e.g, high concentrations, high flow rates). This is supported

by data in this study during Bioreactive Cap B, where the addition of electron donor, albeit at relatively high concentrations, supported complete dechlorination under relative short residence times (1–2 days).
- Careful attention should be provided to accurately characterize seepage rates and contaminant concentrations at sites where contaminated groundwater seeps are present.

In summary, this study examined the conditions governing the implementation of novel subaqueous bioreactive *in situ* caps. Experimental results suggest that the process is feasible provided that sufficient electron donor and contaminant mass fluxes exist in the bioactive cap.

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## Appendix. Supplementary information

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2011.06.022.

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