Journal of Hazardous Materials 213–214 (2012) 311–317

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Electrokinetic-enhanced bioaugmentation for remediation of chlorinated solvents contaminated clay

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ARTICLE INFO

Article history:
Received 5 September 2011
Received in revised form 9 December 2011
Accepted 1 February 2012
Available online 8 February 2012

Keywords:
Bioaugmentation
Electrokinetic injection
Contaminated clay
Bacteria transport
Chlorinated solvents

ABSTRACT

Successful bioremediation of contaminated soils is controlled by the ability to deliver bioremediation additives, such as bacteria and/or nutrients, to the contaminated zone. Because hydraulic advection is not practical for delivery in clays, electrokinetic (EK) injection is an alternative for efficient and uniform delivery of bioremediation additive into low-permeability soil and heterogeneous deposits. EK-enhanced bioaugmentation for remediation of clays contaminated with chlorinated solvents is evaluated. *Dehalococcoides* (*Dhc*) bacterial strain and lactate ions are uniformly injected in contaminated clay and complete dechlorination of chlorinated ethene is observed in laboratory experiments. The injected bacteria can survive, grow, and promote effective dechlorination under EK conditions and after EK application. The distribution of *Dhc* within the clay suggests that electrokinetic transport of *Dhc* is primarily driven by electroosmosis. In addition to biodegradation due to bioaugmentation of *Dhc*, an EK-driven transport of chlorinated ethenes is observed in the clay, which accelerates cleanup of chlorinated ethenes from the anode side. Compared with conventional advection-based delivery, EK injection is significantly more effective for establishing microbial reductive dechlorination capacity in low-permeability soils.

1. Introduction

Chlorinated solvents such as tetrachloroethene (PCE) and trichloroethene (TCE) are among the most frequently encountered organic contaminants in soil and groundwater. Several technologies have been developed to remediate sites contaminated with these chlorinated, volatile organics [1–4]. Bioaugmentation, an eco-friendly approach, is considered one of the favorable methods for clean-up of these sites [5,6]. Microorganisms can degrade organic pollutants due to their metabolic processes and due to their capacity to adapt to inhospitable environments [5]. However, the performances of native microorganisms in an environmental matrix (e.g., groundwater, sediment or soils) may not be sufficient to degrade target contaminants at a reasonable rate. Through addition of specific, pre-grown microbial cultures, the degradation of target contaminants can be enhanced and faster remediation can be achieved. Successful cases of bioaugmentation for dechlorination of chlorinated organics have been reported. A specialized microorganism, *burkholderia cepacia* ENV435, decreased the total mass of chlorinated ethenes in groundwater by 78% within 2 days after bacteria injection [7]. An enhancement on dechlorination was observed for tetrachloride and 1,1,1-trichloroethane when methanogenic enrichment was used in conjunction with Fe(0) [8]. Successful bioaugmentation using KB-1® culture was reported in an in situ pilot-scale groundwater remediation, decreasing concentrations of PCE, TCE and *cis*-1,2-dichloroethene (*cis*-DCE) from several hundred micrograms per liter to below 5 $\mu g\,L^{-1}$ within 200 days after injection [9].

Bioaugmentation is affected by many factors, such as strain selection, microbial ecology, chemical nature and concentration of pollutants, environmental constraints, as well as physicochemical characteristics of the environment [6]. The effectiveness of in situ bioaugmentation for clean-up of chlorinated solvents is highly dependent on the ability to deliver sufficient bioremediation additives (e.g., bacteria and electron donors) to the environmental matrix. However, due to the low-permeability and heterogeneity of certain soil deposits, hydraulic gradient is usually not effective for transport of these reagents from the injection wells to the contaminated zones

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doi:10.1016/j.jhazmat.2012.02.001

[6,10]. Electrokinetic (EK) injection is an alternative method for delivery of bioremediation reagents into low-permeability and heterogeneous deposits. Charged as well as non-charged additives have been successfully injected into soil by ion migration and electroosmosis under electric fields [11–14]. In addition, studies have reported that bacteria could be transported in sand or soil by electrophoresis and/or electroosmosis [15,16], and the bacteria retained their ability to degrade target contaminants during the process.

The primary objective of this study is to evaluate the feasibility of applying electrokinetic processes to facilitate and enhance reductive dechlorination activities of *Dehalococcoides* (*Dhc*) within site-specific low-permeability clay samples. The *Dhc* bacterial strain (in KB-1® culture) is selected because of its unique ability for transforming chlorinated ethenes completely to ethene [9,17,18]. This is the first study to evaluate bioaugmentation of *Dhc* bacterial strain in clay under EK condition. Using clay collected from a contaminated site in Denmark, the study is designed to evaluate the transport and biodegradation activities of *Dhc* under EK, and the enhancing effect of electrokinetics injection on transformation of chlorinated ethenes compared to typical advection-based delivery techniques.

2. Experimental

2.1. Electrokinetic apparatus and setup

The electrokinetic reactor was made of acrylic and consists of a central soil cell and two electrode compartments (Fig. 1a). The central soil cell was rectangular (15 cm in height, 40 cm in length and 5 cm in width) and was filled with the site-specific soil. Acrylic electrode compartments (working volume is 5 L for each compartment) at both ends of the soil cell were used to store the electrolyte and support the electrodes. A gas vent on each sealed electrode compartment was connected to a balloon, which allowed the release of gas pressure resulting from electrolysis during testing. Two high-density polyethylene (HDPE) porous panels (80 μm pore size; 3 mm thick) were used to separate the soil cell and electrode compartments. A separate 1 L tank on a magnetic mixer, as shown in Fig. 1a, was used as a recirculation tank (RT) for the electrolyte and sodium lactate amendment. A peristaltic pump was used to circulate the anolyte and catholyte in the electrode compartments to maintain neutral pH condition and specific lactate concentration. The electrodes (anode and cathode) were graphite plates, each with 150 cm^2 working area. An injection well filled with sand was placed in the center of the soil cell (shown in Fig. 1b) to simulate an injection well configuration for field application. The injection well was made by coring a hole (1 cm diameter; 12–13 cm depth) after the soil was placed in the cell.

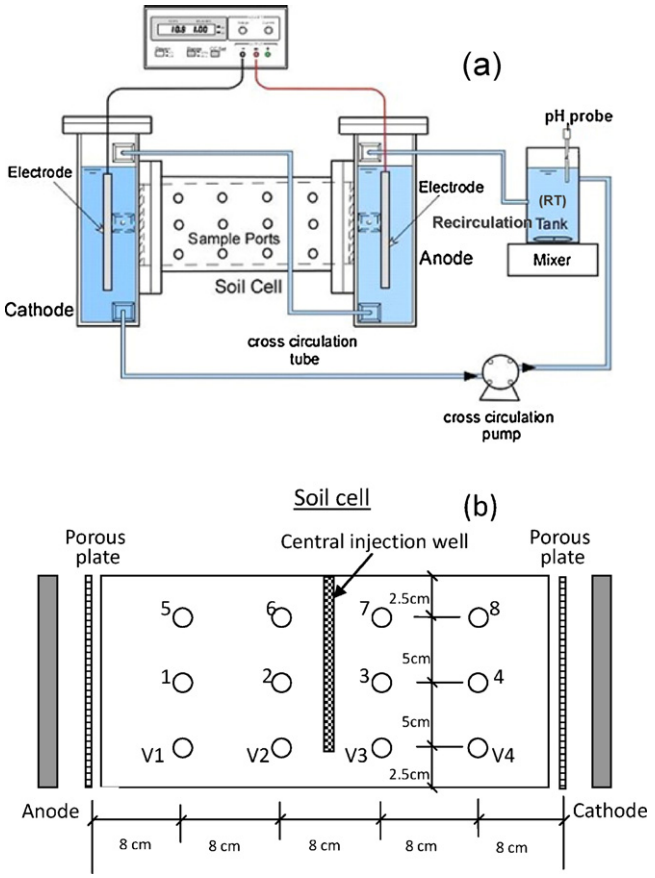

Fig. 1. (a) A schematic of the electrokinetic apparatus and (b) location of the sampling ports (Port-1 to Port-8) and voltage measurement ports (Port-V1 to Port-V4).

Each soil cell had 4 ports (V1 to V4) for voltage measurements and 8 ports (Port-1 to Port-8) for collecting pore-water samples, as shown in Fig. 1b. Stainless steel wires were inserted in the bottom-row ports for measuring the voltage gradient across the reactor. At each sampling port, a porous tube (0.8 cm diameter, 3.5 cm long, made by curling stainless steel screen) was inserted into the compacted soil allowing accumulation of pore-water for sampling. All sampling ports (4 of top-row and 4 of middle-row) were sealed with Swaglok™ nuts and septa, which would allow multiple punctures and maintain air-tight condition.

2.2. Site-specific soil

The site-specific soil samples were collected from a PCE-contaminated site in Denmark. The elevated concentrations of PCE and other chlorinated compounds (TCE and *cis*-1,2-DCE) in the subsurface at this site are associated with past releases of PCE from a former industrial facility located nearby. No other significant contaminants were detected in the soil samples. The soil was stored in air-tight barrels at <4 °C until the cell assembly. The properties of the soil used to fill the soil cells are listed in Table 1. Soil compaction in each cell was completed in 5 layers by a 2.4 kg hammer. To compact each soil layer, the hammer was dropped 40 times from 0.3 m height. After the compaction, around 7 kg soil sample was filled into the cell. The bulk density and porosity of the soil specimens were $2.31(\pm 0.03) \times 10^3$ kg m^{-3} and 0.25 ± 0.01, respectively.

2.3. Experimental procedure

A total of three reactors were constructed for this study. Two reactors (Reactor A and Reactor B) were operated under EK application. The third reactor (Reactor Control) was operated under hydraulic gradient without application of electric field, which served as the control experiment for EK application. The tests under EK application include four stages of operations (P1 to P4, as shown in Fig. 2): (P1) delivery of lactate under EK for 20 days; (P2) injection of KB-1® culture and subsequent acclimation period for 2 days without electric field; (P3) second stages of EK application with simultaneous injection of bacteria and lactate for 60 days (with EK conditions similar to the first stage); and (P4) post-EK incubation (12 days for Reactor A and 78 days for Reactor B).

After the bench test assembly and setup, 11 L solution of 10 g L^{-1} sodium lactate (Sigma–Aldrich, 60 wt% sodium DL-lactate solution) and other nutrient salts (see Table S1 in the supplementary data) were added to the two reservoirs (electrode compartments) and the recirculation tank. In addition, 9 mL of the solution was injected

Table 1
Properties of the soil collected from a site contaminated with PCE.

Items	Level	Note
Soil appearance	Gray black clay with small part of sand (<10 wt%)	Clayey soil
Water content	14 ± 1%	4 subsamples
Total organic carbon (mg per kg dry soil)	Sample 1: 444 Sample 2: 465	Two soil subsamples
pH	7.7	Measured in 20 wt% soil slurry
PCE (mg per kg dry soil)	Sample 1: 464 Sample 2: 142	Two soil subsamples
Lactate ions (mg L^{-1})	Not detectable	The supernatant liquid of the 20% soil slurry
Dehalococcoides copies	2×10^4 vcrA gene copies per gram of wet soil[a]	vcrA testing showed identical level with control sample

[a] The DNA extraction blank performed for this analysis showed 1×10^4 gene copies per gram, which is comparable to the detections in the soil samples. Thus this value is considered potentially false positive for vcrA.

into the central injection well. The power supply was then turned on to begin EK testing. A constant current (38 mA, or a current density of 5 A m^{-2} with respect to the soil cell cross-sectional area) was applied through two graphite electrodes. A peristaltic pump was used to cross-circulate between anolyte and catholyte reservoirs at a flowrate of approximately 25 mL min^{-1}. The liquid in both electrode compartments was always at the same level. No pH-gradient (<0.5) or significant temperature change (<3 °C) was observed between electrolytes from the anode and cathode compartments during the experiments.

KB-1® culture solution (Sirem Lab, Canada) containing 1×10^{11} copies L^{-1} *Dehalococcoides* was used for bioaugmentation. *Dehalococcoides* are a group of microorganisms documented to promote the complete dechlorination of chlorinated ethenes to non-toxic ethene [9]. At sites where *Dehalococcoides* are absent, PCE and TCE dechlorination typically stalls at *cis*-1,2-DCE, despite ample electron donor availability [9]. To bioaugment the soil, the power was turned off and KB-1® culture solution was added to the electrode compartments (5 mL to each electrolyte reservoir) and to the central injection well (1 mL). As a result, the numbers of the bioaugmented *Dhc* are 1.6×10^8 copies per kg wet soil, calculated per mass of the soil in each reactor. After 2 days of acclimation following bioaugmentation, the power supply was turned on to resume the EK operation. The concentration of lactate in the electrolyte was analyzed biweekly during the experiment. If the lactate concentration in the electrolyte was measured to be below 5 g L^{-1}, concentrated sodium lactate solution was amended to the recirculation tank to raise the lactate concentration to around 10 g L^{-1}.

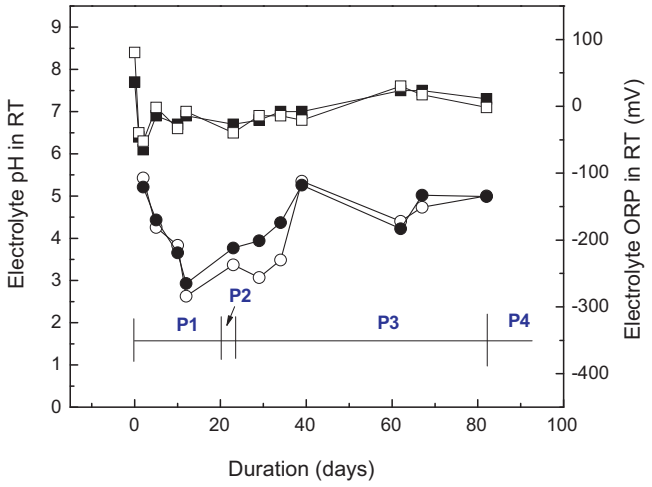

Fig. 2. pH and ORP profiles of the electrolytes in the recirculation tanks (RT) of Reactor A (solid symbol) and Reactor B (empty symbol). Squares represent pH and circles represent ORP measurements. P1: first stage of EK operation (without bioaugmentation); P2: injection of KB-1® culture solution and 2 days of incubation; P3: second stage of EK operation; and P4: post-EK operation.

Pore-water samples were routinely collected from three reactors once pore-water volume was enough for analysis. When testing was completed (after the post-EK incubation stage), clay samples were collected from locations above Port-1 to Port-4 were analyzed.

2.4. Monitoring and analytical methods

pH, oxidation–reduction potential (ORP), and voltage were monitored. Electrolyte was withdrawn from the recirculation tank and the pH and ORP were measured by microprobes (Microelectrodes Inc., Bedford, NH, USA). A voltage meter was used to measure the voltage reading at each of the 4 voltage monitoring ports (V1 to V4 in Fig. 1b). Lactate ion concentration was analyzed by ion chromatography (Dionex DX-120, 1.0 mL min^{-1} sodium carbonate eluent). For each analysis, an aliquot (0.08–0.16 mL) of pore-water or electrolyte was transferred into 5 mL de-ionized water and filtered by 0.45 μm pore size filter paper (Millipore, RW03) prior to loading to the instrument.

Volatile organic compounds (VOCs, including PCE, TCE, *cis*-DCE and vinyl chloride (VC)) and dissolved hydrocarbon gases (DHGs, including ethene, ethane, and methane) in pore-water samples were analyzed by Sirem Laboratory using gas chromatography (headspace method) [17]. For these analyses, 0.2–0.3 mL of pore-water was collected by a gastight syringe from the sampling port, and transferred into a 2 mL crimp-top glass vial prefilled with 1.6 mL of 0.02 mol L^{-1} HCl solution. Chlorinated ethenes in the soil before and after testing were analyzed by GeoLab (Braintree, MA, USA) and Sirem Lab as well. The soil samples were collected into 20 mL crimp-top glass vial, with less than 2 mL headspace. Then these vials were stored with cold packs and shipped to Sirem Lab.

Assessment of microbial quantity was accomplished by a molecular biology method, Gene-Trac® VC, performed by Sirem Laboratory. Gene-Trac® VC is a quantitive polymerase chain reaction (qPCR) test that can be used to quantify the gene that codes vinyl chloride reductase (vcrA), which is responsible for vinyl chloride reduction [17]. Quantity of this enzyme in an environmental media represents the amount of existing *Dehalococcoides*. Both pore-water and soil samples can be analyzed using a DNA extraction process in Sirem Laboratory. For each determination, approximately 0.2 mL pore-water or 5 g soil was used.

3. Results and discussion

3.1. Monitoring of EK operation

Oxidation at the anode generates oxygen gas and acidic electrolyte, leading to an increase in the ORP. Reduction at the cathode generates hydrogen gas and caustic electrolyte conditions, leading to lower ORP. Therefore, although the electrolytes are cross-circulated, the solution in the recirculation tank has to be routinely monitored to maintain suitable survival conditions (5.5–9 pH range

and ORP < −100 mV) for bacteria [9]. As can be seen from Fig. 2, the pH of the circulated electrolyte was well controlled and maintained around 7 throughout the testing duration. The pore-water samples collected from the soil during testing also show that the pH inside the soil was maintained between 7.6 and 8.3 (data not shown), indicating that neither acidic nor basic condition build up in the soils.

The ORP of the solution in the recirculation tank was always maintained below −100 mV due to the presence lactate ions (Fig. 2). Three ORP measurements of the pore-water samples from Reactor A and Reactor B also show that anaerobic conditions with ORP below −100 mV formed inside the soils. After 82 days of EK testing, the total applied voltage for both cells decreased from more than 50 volts to less than 20 V, and the apparent electrical conductivity of the soil specimens increased by 3 times, from 3.9×10^{-4} Simens cm^{-1} (Reactor A), 3.7×10^{-4} Simens cm^{-1} (Reactor B) to 14.3×10^{-4} Simens cm^{-1} for both reactors. The increase in conductivity is due to the transformation of chlorinated ethenes, as well as ions migration and electroosmosis caused by the EK application. More importantly, the EK operation using sodium lactate appears effective in generating and maintaining an anaerobic condition and suitable pH range in the boundary electrolyte and soil, which are essential for the survival of the bioaugmented *Dhc* [9].

3.2. Lactate ions migration

The tests were conducted in four stages. The objective of the first stage was to distribute lactate ions across the soil and maintain neutral conditions prior to the introduction of *Dhc*. Fig. 3 presents the lactate ion concentrations in the pore-water samples collected from the sampling ports of Reactor A. Due to the low permeability of soil, the sampling frequency was primarily dictated by the availability of pore-water in the soil. The first sampling set from all ports was not possible until 10 days of testing. Significant buildup of lactate ions concentration is achieved (Fig. 3) in all ports in Reactor A. Given that the lactate ion front reached Port-1 (32 cm away from cathode) in 10 days under 0.8 V cm^{-1} average voltage gradient, an estimated lactate ion migration rate of 3.2 cm day^{-1} (4.0 cm^2 V^{-1} day^{-1} net effective migration rate) is assumed. This rate is consistent with previous studies that reported transport rates of 3.4 cm day^{-1} for lactate ions, 2.2 cm day^{-1} for citrate, and 2.1 cm day^{-1} for acetate in clay material with hydraulic

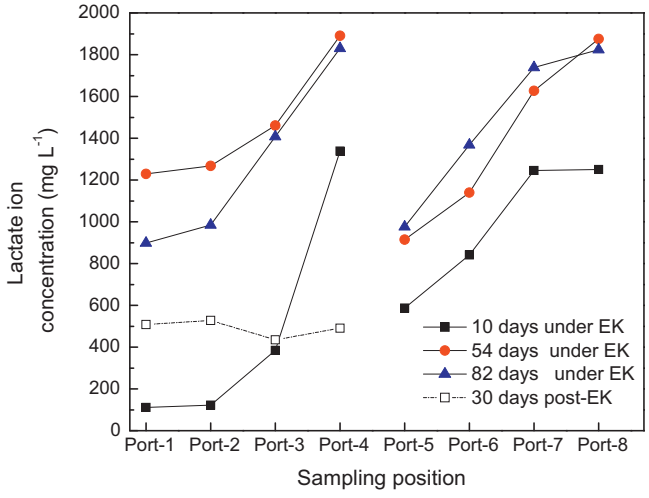

Fig. 3. Distribution of lactate ion concentrations within the soil (Reactor A) at different times.

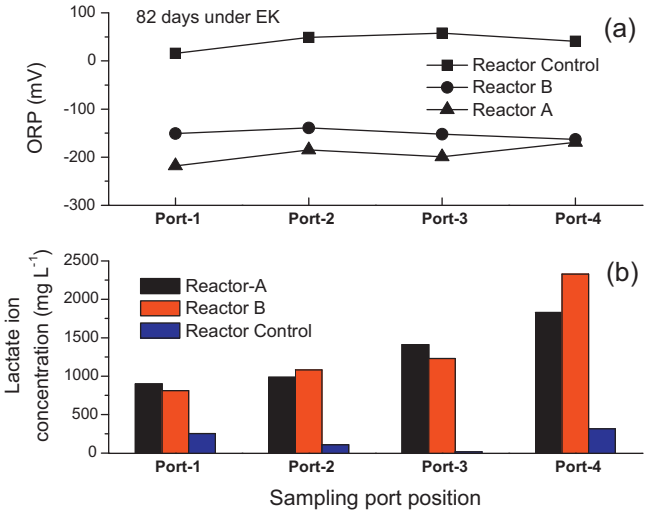

Fig. 4. Comparison of the (a) pore water ORP and (b) lactate ion concentrations in pore water samples collected from Reactor A, Reactor B (82 days under EK operation), and from Reactor Control (82 days after assembly).

conductivity less than 10^{-6} cm s^{-1} (0.086 cm day^{-1}) under current densities ranging from 0.5 to 2.0 A m^{-2} [19,20].

The lactate ion distribution in the soil (Fig. 3) indicates that the transport of lactate ions is governed by ion migration, as the profile shows a decreasing trend from the cathode side to the anode side (e.g., from Port-4 to Port-1). The lactate ion concentration steadily increased in all sampling ports with continuous application of electric current over time. The final lactate concentrations in Reactor A after 82 days of testing were between 800 and 1800 mg L^{-1}, which are equivalent to 9–20% of the boundary lactate ions concentration (8900 mg L^{-1}). After the last stage (post-EK incubation), lactate ion concentrations in Port-1 to Port-4 dropped to around 500 mg L^{-1}, due to the metabolic activities of bacteria. For Reactor Control, the measurements of lactate ions could not be performed at early stages of experiment because of the lack of pore-water availability. The first set of pore-water samples from the control experiment was collected and analyzed after 82 days. A comparison of the ORP and lactate ion concentrations in the pore-water samples collected from the three cells is shown in Fig. 4. The ORP values of the pore-water from Reactor Control were between 16 mV to 58 mV, which is much higher than those of the pore-water from the reactors under EK. The lactate ions concentrations in the control experiment (Fig. 4b) also show very low values compared with those in Reactor A and Reactor B. The comparison in Fig. 4 indicates that the hydraulic gradient was not sufficient to drive the additives or generate a reducing environment in the clay; however, the EK process can enhance lactate delivery and maintain reducing conditions in the soil.

3.3. VOCs and DHGs in pore-water samples

Monitoring of VOCs and DHGs was performed at 3 specific times: 67 days after EK application, 82 days after EK application, and the final one was 12 days after the power was shut off for post-EK incubation. The timing of these sampling events was mostly determined by the availability of pore-water from soil cells, especially for the Reactor Control. The results for Reactor A are presented in Fig. 5. For the first set after 67 days, the distribution of PCE and TCE follows an increasing trend from Port-1 to Port-4. A similar trend is also observed in Reactor B, indicating that the changes are influenced by the electric field (see Fig. S1 in the supplementary data). The distribution of chlorinated solvents in clay under EK is affected by both electroosmosis and ion migration. PCE and TCE molecules would continuously desorb from the soil solids due to

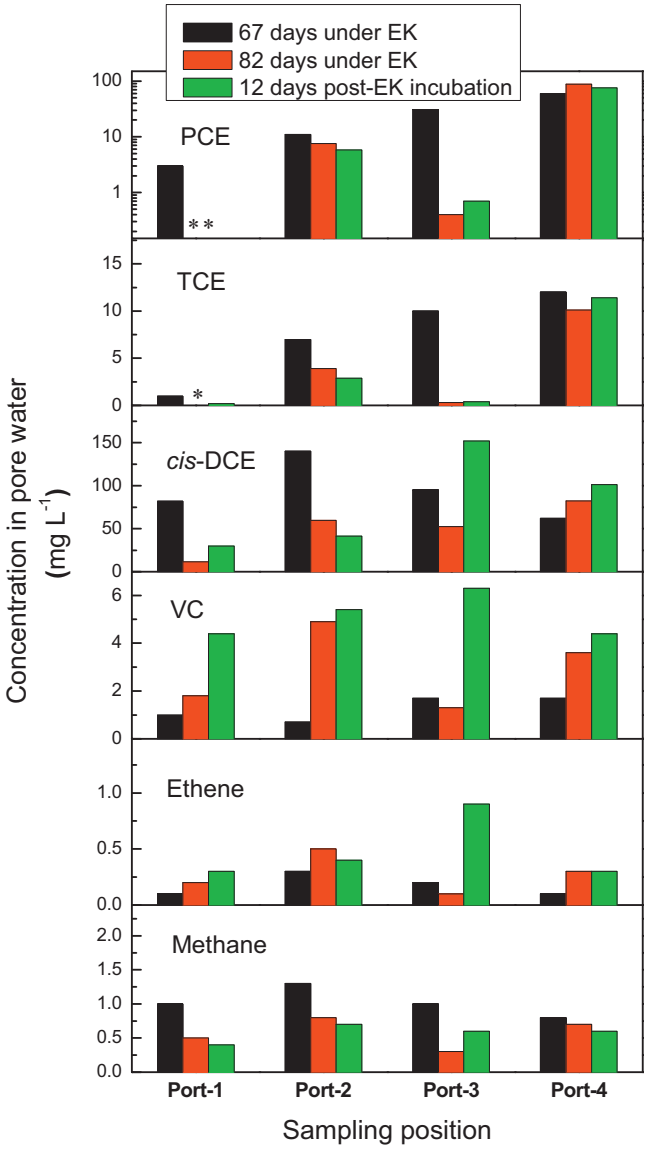

Fig. 5. VOCs and DHGs concentrations in pore water samples collected from Reactor A. The asterisk (*) in the figure indicates that the analytical results are below the quantitative analysis limits (<0.06 mg L^{-1}).

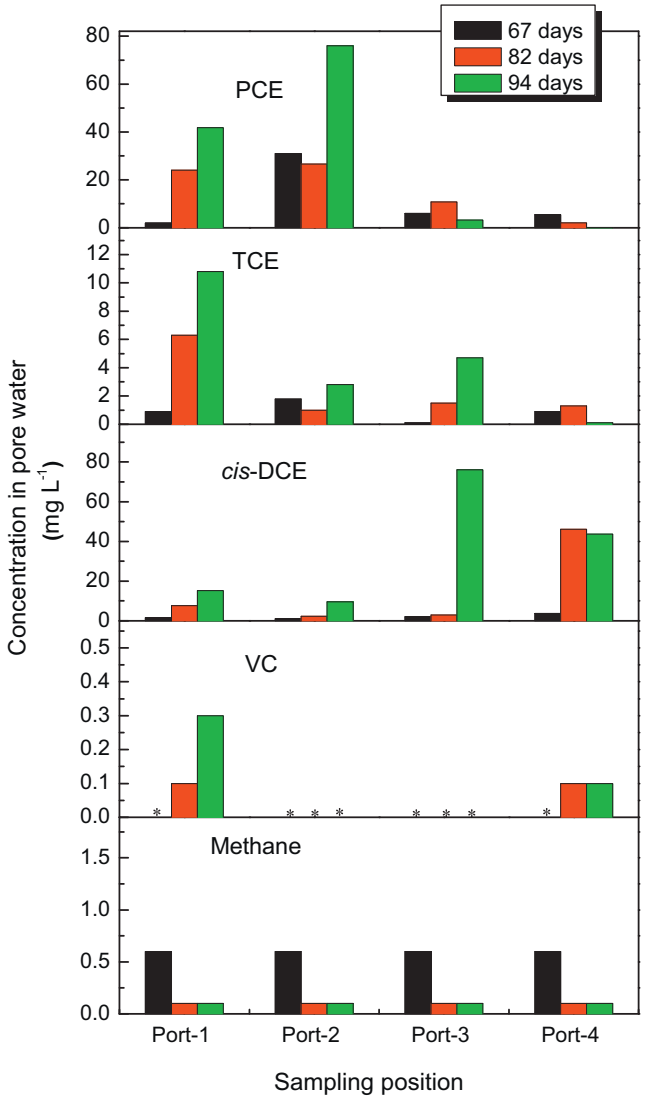

Fig. 6. VOCs and DHGs concentrations in pore water samples collected from Reactor Control. The asterisk (*) indicate that the analytical results are below the quantitative analysis limits.

the movement of pore-water, dissolve in pore-water and transport with moving water by electroosmosis. It is also possible that the process is affected by ion migration. In aqueous solutions, protons (H_3O^+) accumulate around the negatively polarized chlorine atoms of PCE and TCE due to electrostatic attraction, yielding a positively charged ion pair [21]. The positively charged ion pair could migrate from anode toward cathode. The transport by electroosmosis and ion migration, coupled with biological transformation explains the faster dechlorination rate observed in Port-1. No obvious trend can be observed for the distribution of *cis*-DCE. Compared with PCE and TCE, *cis*-DCE shows much higher concentrations, ranging from 30 to 152 mg L^{-1}. This is probably due to the higher solubility of *cis*-DCE in aqueous media and continuous transformation of TCE and PCE.

Without *Dhc* bioaugmentation, the dechlorination of PCE induced by the autochthonous bacteria is expected to stop at DCE (mostly *cis*-DCE), instead of vinyl chloride and non-toxic ethene. Therefore, the detection of vinyl chloride and non-toxic ethene is an important indication of the activity of *Dhc*. After 67 days under EK (45 days after bioaugmentation), VC (0.7–1.7 mg L^{-1}), ethene (0.1–0.3 mg L^{-1}) and methane (0.8–1.3 mg L^{-1}) were detected. Furthermore, an increasing trend in VC and ethene concentrations in pore-water was observed at all ports of Reactor A, indicating that appropriate conditions for complete dechlorination were achieved. More importantly, subsequent increase in VC and ethene concentrations (12 days post-EK incubation) demonstrates the establishment of dechlorination capacity in the soil specimen and continuous biodegradation of the contaminants.

In the control experiment (Fig. 6), the variations of PCE and TCE concentrations in four sampling ports do not follow any clear trend, while *cis*-DCE concentrations increase in all ports. VC was not detectable in Port-2 and Port-3 over the period of 94 days. For Port-1 and Port-4, small amount of lactate ions were available (Fig. 4b) due to the diffusion from boundary electrolyte, thus it is not unexpected that VC is detected at a low level (0.1–0.3 mg L^{-1}). However, because of lack of electron donors or inadequacy of bacteria, ethene was not detected in any of the sampling ports. Since there is no evidence of the activity of *Dhc* in Port-2 and Port-3 (see VC in Fig. 6), it can be inferred that the increasing of *cis*-DCE in these two ports is the result of metabolic activity of the autochthonous bacteria in the soil samples. Small amount of methane (0.1–0.6 mg L^{-1}) was also detected in all ports of control experiment, which can be explained

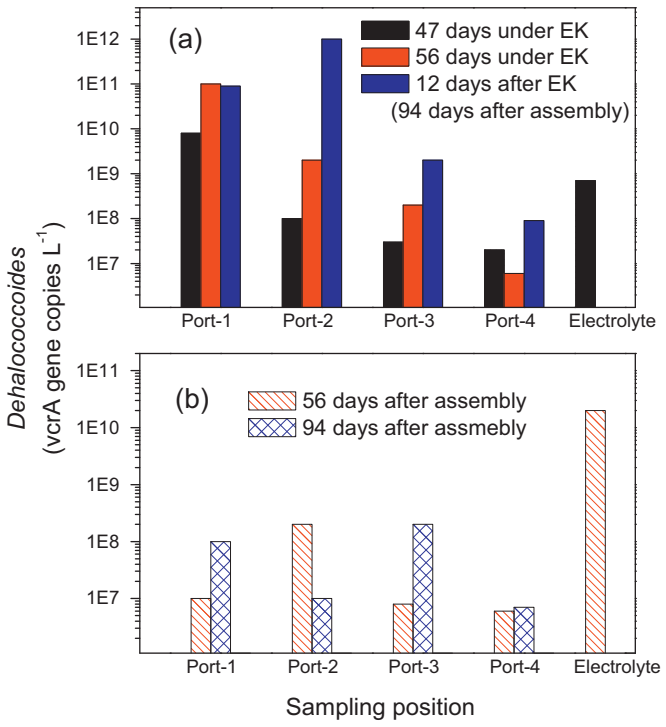

Fig. 7. vcrA copies in pore water samples collected from (a) Reactor A and (b) Reactor Control.

by the metabolic process of the pristine soil organics associated with the autochthonous bacteria like methanogens.

Although 9 mL sodium lactate solution and 1 mL KB-1® culture were injected in the central injection well successively. Neither VC nor ethene was detected in the ports close to the injection well (Port-2 and Port-3) during the 94 days of testing. The results suggest that an injection well, which is commonly used for the injection of amendments into soil matrix, may not be effective for the bioaugmentation of *Dhc* in the control experiment. However, further work is needed for verification.

3.4. *Dehalococcoides* transport

The baseline vcrA soil data suggest that *Dhc* were either absent (potential false positive initial results) or present at very low level (Table 1). Following bioaugmentation of all three reactors, sampling for *Dhc* was first performed before the start of the second stage of EK by collecting electrolyte from the recirculation tank of Reactor A and Reactor B. The vcrA assay of these samples detected 2×10^8 and 5×10^8 vcrA gene copies per liter. The injection volume of KB-1® culture for reactor bioaugmentation was designed, after factoring in the dilution ratio, to achieve a target cell density of approximately 10^8 gene copies per liter. The initial vcrA detections in the electrolyte reasonably reflect the anticipated levels.

Following bioaugmentation, there were 3 sampling events for vcrA assay. Fig. 7a presents the vcrA assay results for Reactor A. Detections of vcrA (ranging from 2×10^7 to 8×10^9 gene copies L^{-1}) were reported for Reactor A in the very first sampling event. Furthermore, the vcrA copies (or *Dhc* copies) decrease along with direction from anode to cathode, which is the direction of the electroosmotic flow. From this observation, it is reasonable to infer that the transport of *Dhc* is associated with electroosmostic flow since the *Dhc* can move along with pore-water. The electroosmostic flow reached Port-1 to Port-4 successively. The sampling ports closer to anode experience longer bacteria growth time and less filtration effect by the soil specimens,

Table 2
Post-test soil analysis (12 days after EK).

Analyte[a]	Sampling position			
	Port-1	Port-2	Port-3	Port-4
PCE (mg kg^{-1})	2.5	30	1.7	51
TCE (mg kg^{-1})	0.2	2.5	0.1	6.6
cis-DCE (mg kg^{-1})	7.5	9.3	14	22
VC (mg kg^{-1})	0.7	0.3	0.3	<0.8
vcrA (gene copies g^{-1})	3×10^7	6×10^7	2×10^7	3×10^6

[a] All the VOCs concentrations are reported by dry soil. The vcrA copy number is reported by wet soil.

thus a descending trend of bacteria distribution from Port-1 to Port-4 is observed. For the second sampling time, levels of vcrA in Reactor A increased and continue to follow the same distribution trend. For post-EK samples, the vcrA copies in Port-2 increased to 1×10^{12} gene copies L^{-1}, or approximately 10 thousand times of the level of the first sample results. In the other ports, the vcrA copies increased by 11 times (Port-1), 67 times (Port-3) and 5 times (Port-4), compared to the first sample results. Concurrent increase of vcrA copies and ethene concentration (see Fig. 5) confirms that EK process was effective in injection of *Dhc*, and the *Dhc* were actively growing and dechlorinating VOCs to ethene.

The first detection of vcrA in the control experiment ranged from 6×10^6 to 2×10^8 gene copies L^{-1} in all four ports, as shown in Fig. 7b. These vcrA levels are higher than the observed baseline soil data, but typically lower than those observed in Reactor A at the same sampling date and in the boundary electrolyte of Reactor Control. The final data collected for Reactor Control show similar detections of vcrA as the first sampling event, indicating that *Dhc* were not actively growing in Reactor Control. Monitoring of DHGs in the control experiment (Fig. 6) did not show detectable levels of ethene throughout the test duration, supporting the vcrA data.

3.5. *Post-test soil analysis*

Soil specimens were collected from Reactor A 12 days after the electric power was shut down and were analyzed for VOCs and vcrA concentrations (Table 2). Compared with initial concentrations in the soil (Table 1), the PCE concentrations decreased from several hundreds to several dozens of mg g^{-1} at all ports. Due to the combined action of electroosmosis and bioaugmentation, the soil at Port-1 had the lowest PCE concentration. The distributions of other chlorinated organics along the sampling ports reasonably correspond to the distribution of VOCs in the pore water. The vcrA data in Reactor A show a range of 3×10^6–6×10^7 gene copies g^{-1}, which is higher than the vcrA levels in the control experiment (1×10^4 and 1×10^6 in Port-2 and Port-3, respectively). Reactor B was sampled and tested 78 days after the shutting down of electrical power. A decrease in VOCs concentrations (data not shown) and a considerable growth of vcrA copies (from 4×10^{11} to 2×10^{12} gene copies g^{-1}) are observed in the soil samples.

Several observations can be derived based on the above data. The EK process is able to deliver the *Dhc* effectively in this low-permeability soil sample. The bioaugmented *Dhc* culture can survive and establish under EK conditions in clay when sufficient electron donors are delivered simultaneously. In spite of electrolytic oxidation at the anode, the electrical field and electrochemical reactions do not appear to negatively impact the bioaugmented *Dhc*. In addition, the dechlorination activity of the bioaugmented *Dhc* was not inhibited by the applied electrical field, since increased levels of VC and ethene are detected at the interior sampling ports of the reactors under EK testing. In this study, the EK operating conditions,

such as the current intensity and boundary lactate concentrations, basically follow those reported in the literature (such as lactate transport in sand and clay [20]). However, for the implementation of the process at a real site, the operating conditions and EK treatment time may be optimized to decrease energy consumption. For example, the bacteria can be added once the current is applied, and the processing time can be varied according to the mass of the electron donor required for a complete dechlorination. Based on this work, and considering the unanticipated challenges that a real site may pose, a pilot scale EK enhanced bioaugmentation for the in situ remediation of a PCE-contaminated site is underway in Denmark. The results will be reported once the study is completed.

4. Conclusions

The study demonstrates that electrokinetic transport can be effective for delivering bioremediation reagents to contamination hotspot in clayey soil. Under 5 A m^{-2} current density, *Dehalococcoides* bacterial strain and lactate ions are successfully delivered and a complete dechlorination of PCE are achieved. An electrolyte cross-circulation system can maintain neutral pH and negative ORP value (<−100 mV) during EK application. The lactate ion transport in the site-specific soils is mainly controlled by ion migration and its transport rate is approximately 3.2 cm day^{-1}. The site soil can be effectively bioaugmented with KB-1® culture, and the delivered *Dhc* can survive, grow, and promote effective PCE dechlorination to ethene under and after electric current application. The distribution of *Dhc* within the soil cell suggests that *Dhc* transport is primarily driven by electroosmosis. In addition to the biodegradation effect from the bioaugmented *Dhc*, an EK-driven movement of chlorinated compounds inside the soil cell is observed. This movement accelerates the clean-up of contaminants near the anode side. Without the need of long-term EK application, an EK-enhanced bioaugmentation (EK-bio) could overcome a variety of technical challenges that conventional EK or bioremediation processes may face. This process could be considered a feasible in situ remedial option for low permeability zones that are contaminated by chlorinated solvents.

Acknowledgments

The work conducted for this project is funded by the Capital Region of Denmark and by award number P42ES017198 from the National Institute of Environmental Health Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Capital Region of Denmark, the National Institute of Environmental Health Sciences or the National Institutes of Health. Xuhui Mao also thanks NSFC (51108353) for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.02.001.

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