Electrokinetic transport of bacteria

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Abstract

The remediation field is in need of a simple, safe, and cost-effective technology that directly treats contaminants sorbed to aquifer solids. In the absence of an indigenous microbial population competent to degrade the contaminants of concern, bioaugmentation, or injecting competent degradative bacteria into the subsurface has been investigated. A major problem encountered with bioaugmentation is the limited dispersion of bacteria injected into contaminated aquifers: bacteria often adhere strongly to solid surfaces. In the absence of a strong hydrogeological gradient the organisms remain localized at the origin of injection, resulting in fouling of wells and inadequate dispersion of degradative bacteria. The ability to directionally transport bacteria away from injection sites and into zones of contamination would be advantageous to bioaugmentation approaches used for in-situ remediation.

Bench-scale model aquifers were used to test electrophoresis as a tool for bacterial dispersion in situ. Preliminary studies with several strains demonstrated that the net negative surface charge of bacteria in a solution at neutral pH caused electrokinetic transport of the cells through sand towards the anode in a dc electric field. Subsequent experiments were conducted in a variety of porous media with a trichloroethylene (TCE) degrading, adhesion-deficient variant of *Burkholderia cepacia* G4 (1CB). 1CB was directionally transported through sand, soil, and aquifer sediment at rates ranging from 1.6 to 6 cm h depending upon the porous media tested, over distances up to 0.4 m. Transport of the wild-type G4 through sand and sediment in response to a hydraulic gradient is severely retarded in relation to the adhesion-deficient 1CB, whereas electrokinetic transport rates are identical for the two strains. Experiments performed with TCE-contaminated sediment suggest that 1CB retains its ability to degrade TCE during electrokinetic transport. © 1997 Elsevier Science B.V.

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1. Introduction

The application of direct current between two electrodes in soil has two effects: electroosmosis, the movement of interstitial water toward the cathode; and electromigration, the movement of ionic species, both soluble and particulate, to the oppositely charged electrode. The movement of charged particles to the oppositely charged electrode is also known as electrophoresis [1].

Electroosmosis, or the movement of water due to the application of an electric current in very fine-grained, low permeability soils (micrometer-size or smaller pores) can be used to remove dissolved contaminants. Contaminants with limited solubility or those that sorb to surfaces cannot be remediated by electroosmosis [2]. This process has also been proposed for creating electrode fences within which water is electroosmotically driven to confine contamination plumes [3]. This movement of pore water towards the cathode does not occur in higher permeability soils because it is dependent on the charge density of the soil itself. Electrokinetics or electromigration, however, can occur in all soils because it is independent of the charge density of the soil and dependent on the charge density of the entity being moved, i.e. metals, radionuclides, charged organics, and, in this case, bacteria. During what is termed 'unenhanced electrokinetics' the electrochemistry associated with this process produces an acid front that moves from the anode to the cathode [4,5]. The relative shifts in pH at the electrodes and migration of the acid front will depend upon the buffering capacity of the soil or groundwater system. Under certain conditions, this low pH front can help to mobilize metals that are being remediated [6]. The term 'multicomponent species transport' has been used to describe the electroosmotic transport of species solubilized by this acid front [7]. In some cases, however, it is necessary to prevent the development of these pH excursions at the electrodes; therefore, a great deal of research has been done on ways to 'condition' the electrodes [4]. For example, pH excursions can cause precipitation of certain species in soil, hindering recovery of the contaminants [8]. In our application of electrokinetics, pH control is very important because extremes in pH could kill degradative bacteria introduced for remediation.

Electromigration of ions is faster by at least one order of magnitude than their electroosmotic mobility, and does not require saturated, low porosity soils. In experiments designed to investigate the application of electrokinetics to remediate a chromium-contaminated site, an anionic organic dye was used as a chromium analog. The highest electromigration rate of the organic dye tested was seen in the driest sand (7% water by weight [1]), effectively broadening the applicability of this technology to include vadose zone contamination. Electroremediation has been used in the field mostly for inorganic contaminants. Successful field remediations in the Netherlands have used electrokinetics to remediate soils contaminated with lead, copper, zinc, cadmium and arsenic [6,9].

Remediation of aquifers contaminated with hydrophobic organic chemicals has met with limited success using currently available treatment technologies. Pump-and-treat methods which bring contaminated groundwater to the surface for treatment do not treat the source of the problem, the mass of contaminant adsorbed to the surface of the sediment. Without removing the source of contamination, groundwater at these sites is

continuously recontaminated for long periods of time by slow desorption of the contaminant from the aquifer sediment. The effectiveness of in-situ bioremediation is dependent upon the ability of the biocatalyst to come into contact with and transform contaminants into non-toxic substances. With a bioaugmentation approach, success depends in part on the ability to direct the movement of microorganisms through the subsurface. Another important parameter is the time required for the microorganisms to reach the full extent of the contaminated zone. Bacteria introduced to a site by injection can migrate through the subsurface on their own or in response to natural or imposed hydrologic gradients. Unfortunately, this movement can be blocked by endogenous aquifer conditions, limiting biocatalyst contact with contaminated surfaces. Therefore, it is advantageous to have the ability to direct the movement of bacteria in situ, both laterally and opposite to the groundwater flow direction.

Charged polymers on the bacterial cell surface contain ionizable groups that result in an amphoteric surface. This surface carries a net negative charge at high pH and a net positive charge at low pH [10]. At neutral pH, most bacteria act as negatively charged particles, which dictates their movement towards the anode in an electric field [11]. Electrophoretic mobility (EPM), a measure of the net surface charge of bacteria, has been used to study a number of effects on surface properties. Among the conditions that have been found to alter the EPM of specific bacterial strains are: surfactants [12], growth phase, growth medium, pH, antigens, antibiotics and bacteriophage infection [11], and heavy metals [13]. EPM has also been used to predict bacterial interactions with surfaces (i.e. adhesion [14]). Species differences in EPM have been used to separate mixtures of strains into discrete electrophoretic bands that correspond to relatively pure cultures [10].

In many cases of groundwater pollution, in situ bioremediation may be the only cost-effective, time-efficient treatment technology. In environments where the indigenous microbial population is incapable of degrading the compounds of concern, it may be desirable to add degradative bacteria throughout the contaminant plume. In the absence of a favorable groundwater gradient, the problem becomes one of installing enough injection and recovery wells to draw nutrients and/or metabolically active bacteria throughout the contaminated zone. Electrochemical remediation has great potential as an in situ treatment technology for removing a wide range of contaminants from the subsurface, including both metals and organics [9,15,16]. Our work has shown that we can use this same technology to directionally transport microorganisms through sediment and soil. This application for electrochemical remediation differs from others in that it is an in situ destruction technology and the entity being transported are strains of bacteria capable of degrading organic contaminants in the groundwater and adsorbed to aquifer solids [17].

2. Methods and materials

2.1. Bacteria

For preliminary experiments, bacteria grown to exponential phase at 30°C in LB (Luria–Burtani [18]) broth were used to test the feasibility of this approach. The strains

used in these studies included a non-motile soil isolate *Pseudomonas fluorescens* (Pf0-5), two E. coli strains JM83 and W3110, and *Burkholderia cepacia* G4 (G4; formerly *Pseudomonas cepacia* G4; [19]). In later experiments, an active trichloroethylene (TCE) degrading culture of adhesion-deficient *Burkholderia cepacia* G4 (1CB; [20]) was maintained in a Cytolift® bioreactor operating as a toluene chemostat containing 0.6 l of basal salts medium (BSM [21]). Toluene vapor was supplied with air sparged into the bottom of the reactor as the sole carbon/energy source and limiting nutrient. Toluene or phenol is required by G4 as a cometabolite for TCE degradation [22]. TCE degradation by G4 and 1CB has been studied extensively [20,23]. Aliquots were removed from the reactor and diluted in BSM to the desired cell density prior to inoculating the model aquifers. 1CB is resistant to the antibiotic kanamycin due to a transposon insertion (Tn5) used to induce the adhesion-deficient phenotype [20].

2.2. Model aquifers

2.2.1. Small model aquifer

Small-scale model aquifers were constructed by filling the tray of a horizontal submarine gel electrophoresis apparatus (Bio-Rad Mini-Sub Cell Electrophoresis Box #170-4307, Hercules, CA) with 'model' aquifer sediment and adding buffer to saturate the sediment. The length of this box and the distance between the two electrodes is 15 cm, with a 10 cm long tray containing the sediment. The width and depth of the tray are 6.5 and 1.5 cm, respectively. Fiberglass screening was affixed to the open ends of the gel trays with silicon adhesive to contain the sediment.

2.2.2. Large model aquifer

A larger-scale model aquifer (Fig. 1) was constructed to measure electrophoretic transport of bacteria over longer time frames and distances. To reduce the effects of pH gradation caused by H^+ and OH^- generation at the cathode and anode during extended electrophoresis, reservoirs containing one liter of buffer were used to recirculate the buffer surrounding each electrode. A single peristaltic pump with tubing cartridges was used to recirculate the anode and cathode buffers at a rate of $2 \, ml \, min^{-1}$. In an attempt to prevent a hydraulic gradient, tubing used to remove buffer from the model aquifer was slightly larger in diameter than that used to return the buffer, and the ends of the anode and cathode withdrawal tubes were positioned at the same depth, just above the top of the aquifer solids. Sufficient buffer was added to cover the aquifer solids, with approximately 0.5 cm of buffer above the level of the sediment.

2.3. Aquifer materials

The porous media utilized in this study as model aquifer sediment were a coarse-grained Ottawa sand (20–30 mesh; ~ 750 μm diameter; Fisher Scientific), fine-grained play sand obtained from a local hardware store (50–100 mesh; 70–350 μm diameter), two well characterized soils: (1) Adelphia sandy loam (72% sand, 14% silt, 14% clay, 1.4% organic matter, 0.8% carbon) and (2) Quakertown silt loam (20% sand, 60% silt, 20% clay, 3.9% organic matter, 2.2% carbon), and a very fine sand/silt/clay (< 160 μm
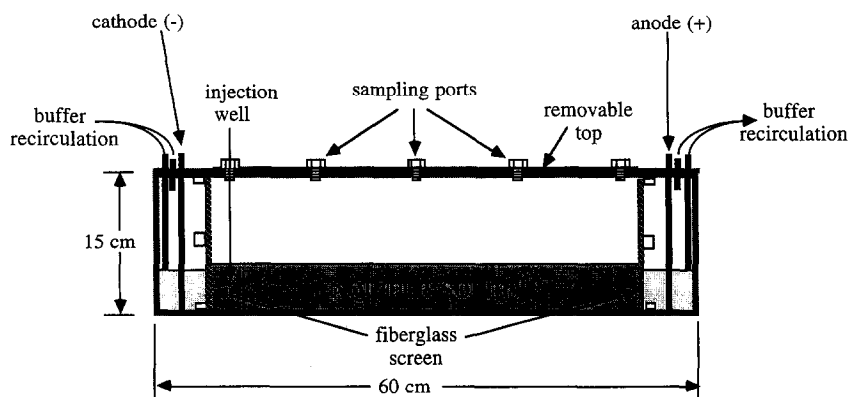
LARGE MODEL AQUIFER

Fig. 1. Large-scale model aquifer, constructed from 1/4 inch acrylic (60 cm × 30 cm × 15 cm ID) with stainless steel electrodes traversing the width of the buffer reservoir. The aquifer solids were partitioned from the reservoirs by a fiberglass screen mounted on an acrylic frame. A peristaltic pump was used to recirculate the buffer surrounding the electrodes at a rate of approximately 2 ml min^{-1}.

average diameter) aquifer material obtained from the Environmental Protection Agency Laboratory at Ada Oklahoma (US EPA). Tris acetate buffer (TA; 0.045 M; pH 7.0) and tris borate buffer (TB; 0.045 M; pH 7.0) solutions [18] used in the model aquifers were prepared from sterilized groundwater.

The sand was prepared by sterilization in an autoclave (20 psi, 120°C) twice for 90 min each time. The soils and sediments were sterilized by the same method, air dried, crushed and stored in sterile glass jars. For large-scale model aquifer experiments, approximately 7500 cm^3 of sand was poured into the model aquifer containing TA buffer 24 h before beginning the experiment.

2.4. Transport monitoring

2.4.1. Small model aquifer

In preliminary experiments, cells from an exponentially growing culture of bacteria were introduced in a small volume (20 μl) to the center of the sand bed (Ottawa) in the small-scale model aquifer. Electric current from a FisherBiotech FB420 power supply at 35–50 V was run for 2–4 h. One gram of sand was then sampled from the cathode end of the sand bed, the middle, and the anode end. Each sand sample was mixed rapidly by vortex for 1 min in 9 ml of phosphate buffered saline and the supernatant was serially diluted for colony plate counts to determine the number of cells at each position. Control experiments were run in an identical manner without an electric current.

The rate of movement of several bacterial strains through the model aquifer was determined by introducing a small volume (100 μl) of an exponentially growing culture at the cathode end of the sand bed and then taking 100 μl samples of the interstitial

buffer every half hour at the anode end of the sand bed 6 cm away. Transport in the Adelphia and Quakertown soils was monitored by taking interstitial samples at 3 cm and 6 cm from the cathode injection point. All samples were diluted in phosphate buffered saline and plated on the appropriate media for colony counts. Except for preliminary experiments designed to determine transport rates at different voltages, all experiments in the small model aquifers, including TCE degradation experiments, were run at $10\,V\,cm^{-1}$.

2.4.2. Large model aquifer

All experiments in the large model aquifer were conducted with 1CB. Bacterial inocula were injected to a depth equal to the center of the saturated aquifer material near the cathode end of the large model aquifers and dc voltage was applied at time zero. Transport was monitored by removing samples (0.3 ml) of interstitial fluid from various locations downstream from the point of inoculation, and performing serial dilutions in phosphate buffered saline. Dilutions were plated onto Luria–Burtani agar supplemented with kanamycin sulfate (Sigma Chemical Co.) at $25\,\mu g\,ml^{-1}$. After 48 h of incubation at 30°C, colonies were counted to determine colony forming units per milliliter ($cfu\,ml^{-1}$).

The experiments performed in the large model aquifer were run at $4\,V\,cm^{-1}$. The rate of transport in all experiments was calculated from the number of hours for the highest concentration of cells to reach the anode sampling point, divided by the distance traveled.

2.5. TCE degradation in model aquifers

Studies on TCE degradation were conducted in the small model aquifers with sand and sediment that had been sterilized by autoclaving (125°C, 20 psi) twice for 90 min each time and then 'pickled' in 1000 ppm TCE (Aldrich Chemical) in deionized/distilled water for at least one month. This treatment effectively adsorbs TCE onto the surface of the sand. Prior to electrophoresis, the TCE-contaminated sediment was washed with one pore volume of TA buffer (pH = 7.0) and placed into two small model aquifers connected to the same power supply. One model aquifer was used as a control to determine non-degradative losses of TCE due to volatilization, dilution, desorption, and transport. At the start of the experiments, 1CB (3×10^8 cells) was added to the aquifer sediment in a line perpendicular to the electric field 1 cm from the cathode end of the gel tray. After 3 h of electrophoresis, a second dose of 3×10^8 cells was added at the cathode end of the sediment. After 6 h of electrophoresis, three 1 g samples of aquifer material were removed from the cathode, center, and anode portions of the model aquifer, placed in 20 ml serum bottles, and crimp-sealed with Teflon-lined rubber septa. TCE was extracted from the aquifer material by adding 2.5 ml of pentane and shaking the bottles (150 rpm) horizontally for 1 h at room temperature (22°–25°C). Concentrations of TCE were determined by direct injection into a Varian 3400 Gas Chromatograph equipped with an electron capture detector.

2.6. pH effect on transport

To test the effect of electrolyte pH on transport rate and recovery through aquifer solids, TA buffers at pH 5.5, 6.5, 7.5 and 8.5 were tested with Ottawa sand in the small

model aquifers. An inoculum of $100\,\mu l$ of 1CB at an optical density (OD_{550}) of 2.0 $(4 \times 10^8\,\text{cfu ml}^{-1})$ was added to the cathode end of the model aquifer and a $10\,\text{V cm}^{-1}$ dc electric field applied. Samples were collected at a point 6 cm away at the anode end of the sand bed every hour for 6 h and plated, as described above, for enumeration.

3. Results

Preliminary experiments demonstrated that, in the absence of a dc electric field, bacteria failed to migrate through Ottawa sand in the small model aquifers. In experiments designed to determine the direction of bacterial electromigration, four different bacterial strains; a non-motile *Pseudomonas fluorescens* (Pf0-5), two *E. coli* strains (JM83 and W3110), and *Burkholderia cepacia* G4, exhibited unidirectional movement towards the positive electrode (anode) in an electric field. Experiments designed to determine the rate of electromigration were performed with the two *E. coli* strains and both the wild-type and adhesion-deficient mutant of *B. cepacia* G4. Under identical conditions, both *E. coli* strains were transported at a slower rate than the *B. cepacia* strains, but both the wild-type and mutant G4 (1CB) had identical transport rates in Ottawa sand (Table 1). Most experiments resulted in a sharp peak of bacteria, representing the majority of the cells being transported together. However, in experiments with *E. coli* W3110, there were two rates of migration. At $5\,\text{V cm}^{-1}$, the major peak of bacteria passed the 6 cm point at 3.5 h, but there was a second peak of bacteria that passed at 4.5 h. This same type of pattern was seen when the same cells were subjected to $10\,\text{V cm}^{-1}$, with a peak at 2.5 h and another at 3.5 h. Transport rates for W3110 in Table 1 were calculated from the time the first peak of bacteria reached the 6 cm sampling point.

Several electrolytes were tested for their buffering capabilities and ability to support viable bacteria. Most of the electrolytes tested were eliminated due to poor buffering capacity in an electric field which resulted in rapid pH partitioning and death of the introduced bacteria. Trisma® base, in TA and TB buffers, was selected for these experiments due to its buffering capacity, electrolyte properties, and low cost for large-scale use.

Table 1
Electromigration rates for different bacterial strains in the small model aquifer. The model aquifer sediment in these experiments was Ottawa sand

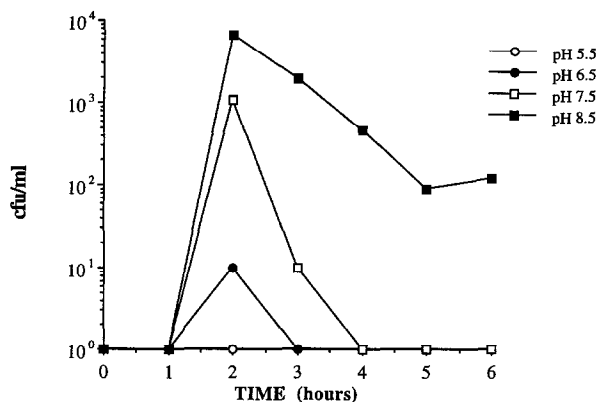| Voltage (V cm^{-1})	Bacterial strain	Transport rate (cm h^{-1})	Buffer
5	*E. coli* W3110	1.7	0.045 M TB
10	*E. coli* W3110	2.4	0.045 M TB
10	*E..coli* JM83	1.4	0.045 M TB
5	*B. cepacia* G4	1.2	0.045 M TB
10	*B. cepacia* G4	4	0.011 M TB
10	*B. cepacia* G4	2.4	0.022 M TB
10	*B. cepacia* G4	3	0.045 M TB
10	*B. cepacia* 1CB	3	0.045 M TB
10	*B. cepacia* 1CB	3	0.045 M TA

Fig. 2. Transport of *B. cepacia* 1CB through Ottawa sand in the small model aquifer. Identical experiments were run with TB buffer adjusted to four different pH values: 5.5 (O), 6.5 (●), 7.5 (□) and 8.5 (■).

In experiments designed to test the effect of pH, the rate of electrokinetic transport was the same at pH 6.5, 7.5 and 8.5 (3 cm h^{-1}). The recovery of cells at pH 8.5, however, was approximately 3 orders of magnitude higher than at pH 6.5 and approximately 6 times higher than at pH 7.5. Cells were not recovered at the 6 cm sampling point during a 6 h sampling period for experiments run at pH 5.5 (Fig. 2).

Reproducible results were obtained when transporting 1CB through Ottawa sand in the small model aquifers (Fig. 3A; Table 1). Electrokinetic transport was demonstrated at rates of approximately 3 cm h^{-1} when a 10 V cm^{-1} voltage gradient was applied in TA or TB buffer at 0.045 M. These experiments were repeated using the finer-grained play sand. Transport rates were consistently higher in play sand (Fig. 3B) than in the Ottawa sand (6 cm h^{-1} vs 3 cm h^{-1}, respectively) when imposing the same voltage gradient. Transport through soil (Fig. 3C and 3D) was also demonstrated using a 10 V cm^{-1} voltage gradient. Both transport rate and recovery of cells were lower in the Adelphia and Quakertown soils than in sand. The rate of electromigration was 1.5 cm h^{-1} for the Adelphia (Fig. 3C) and 1 cm h^{-1} in Quakertown soils (Fig. 3D). In these soils, however, low cell recovery at the 6 cm sampling point precluded quantification; therefore, calculation of the transport rate was done from the 3 cm sampling point.

The large model aquifer (Fig. 1) provided evidence that electrophoresis of bacteria could be conducted on a larger scale. During the prolonged time frames used in these experiments a significant pH gradient developed as a result of electrolysis reactions. The incorporation of a buffer recirculation system greatly reduced this effect. A transport rate of 3.3 cm h^{-1} in Ottawa sand was obtained with a 4 V cm^{-1} voltage gradient (Fig. 4). There was no evidence of a significant hydraulic gradient in these model aquifer experiments; however, it is necessary to measure the electric gradient along the path between the two electrodes in order to eliminate the possibility of a hydraulic gradient [24]. These measurements were not made in this study.

Results from the TCE degradation experiments showed a significant decrease in TCE in the presence of 1CB (Figs. 5 and 6). Plate counts of interstitial samples demonstrated that degradation of TCE did not affect 1CB transport rates (data not shown). Control
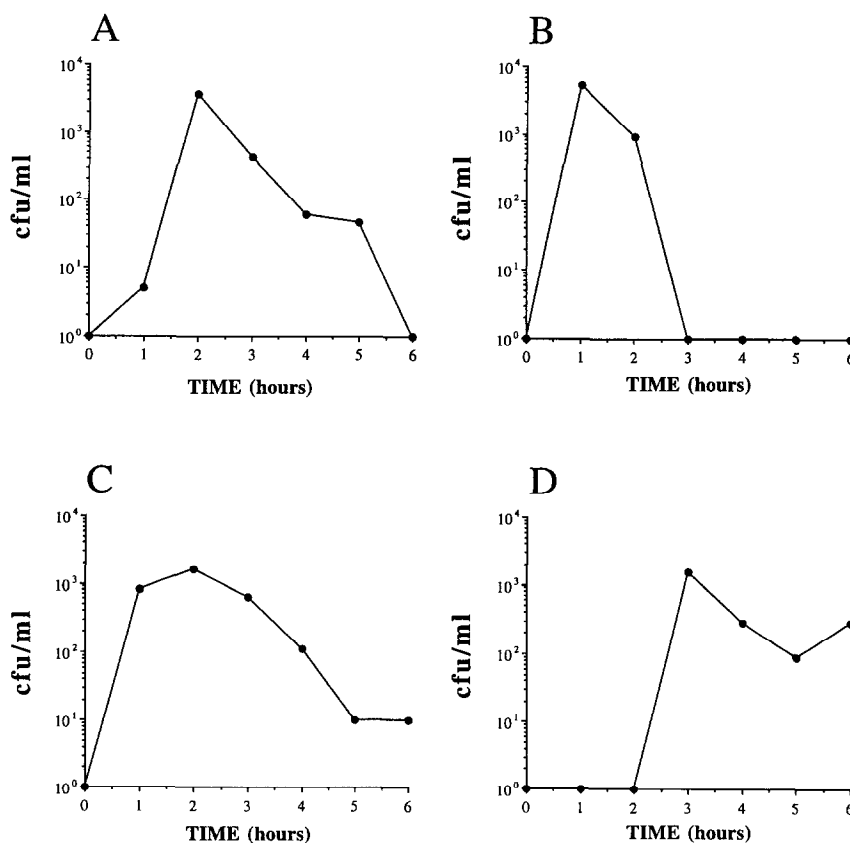
Fig. 3. (A) 1CB transport through Ottawa sand in the small model aquifer. A 0.1 ml inoculum of 1CB at $OD_{550} = 1.2$ was injected into the cathode end of the sand bed and a $10\,V\,cm^{-1}$ (12 mA) DC current applied. Cells were recovered 6 cm away and demonstrated a transport rate of $3\,cm\,h^{-1}$. (B) 1CB transport through play sand in the small model aquifer. A 0.1 ml inoculum of 1CB at $OD_{550} = 2.0$ was injected into the cathode end of the sand bed and a $10\,V\,cm^{-1}$ (40 mA) dc current applied. Cells were recovered 6 cm away and demonstrated a transport rate of $6\,cm\,h^{-1}$. (C) and (D) 1CB transport through Adelphia (C) and Quakertown (D) soils in the small model aquifer. A 0.1 ml inoculum of 1CB at $OD_{550} = 2.0$ ($8.6 \times 10^8\,cfu\,ml^{-1}$) was injected into the cathode end of each soil bed and a $10\,V\,cm^{-1}$ (Adelphia, 50 mA; Quakertown, 70 mA) dc current applied. Cells were recovered 3 cm away and demonstrated transport rates of $1.0\,cm\,h^{-1}$ (Adelphia) and $1.5\,cm\,h^{-1}$ (Quakertown).

experiments were run without bacteria to measure abiotic losses of TCE from the aquifer solids being exposed to an electric current. It was found that TCE moves in an electric current in the direction opposite that of the bacteria and approximately one order of magnitude slower. TCE is also volatile and there were some volatility losses over the course of these experiments. These control experiments demonstrated that the magnitude of abiotic losses did not preclude the measurement of microbial degradation in this system. A control model aquifer without bacteria was run each time we ran an experiment with added bacteria, allowing measurement of the loss of TCE attributable to
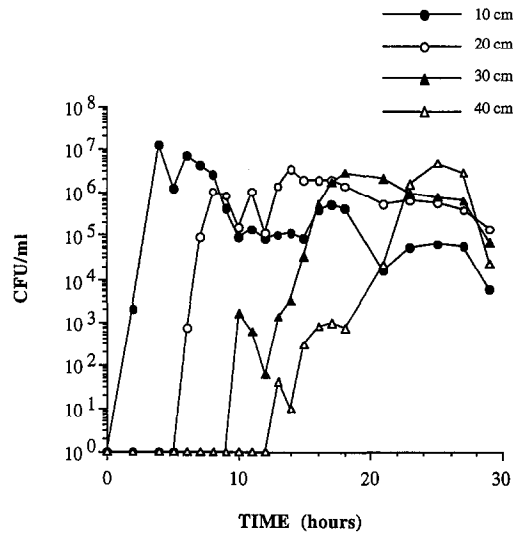
Fig. 4. 1CB transport through Ottawa sand in the large model aquifer. A 5.0 ml inoculum of 1CB at $OD_{550} = 3.0$ was injected into the cathode end of the model aquifer and a $4\,V\,cm^{-1}$ (15 mA) dc current applied.
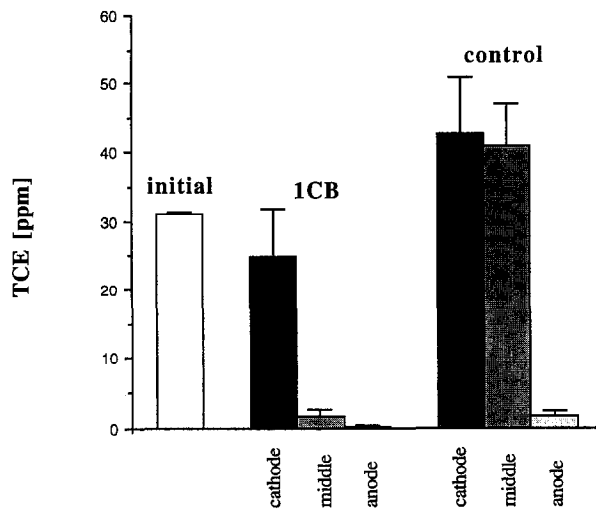

Fig. 5. TCE removal from contaminated Ottawa sand in an electric field. Error bars represent the standard deviation of triplicate samples taken from the cathode, middle, and anode portions of the small model aquifers. The initial concentration was derived from samples taken prior to loading the gel tray with sand. The experiment utilized a $10\,V\,cm^{-1}$ voltage gradient and two inocula of 0.3 ml of 1CB at $OD_{550} = 2.0$ ($1.0 \times 10^9\,cfu\,ml^{-1}$) dosed at time zero and again after 3 h.
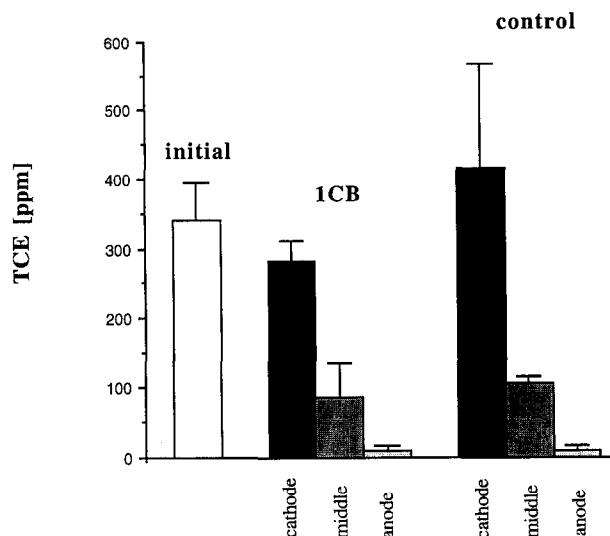
Fig. 6. TCE removal from contaminated EPA sediment in an electric field. Error bars represent the standard deviation of triplicate samples taken from the cathode, middle, and anode portions of the small model aquifers. The initial TCE concentration was derived from duplicate samples taken prior to loading the gel tray with sediment. The experiment utilized a $10\,V\,cm^{-1}$ voltage gradient and two inocula of 0.3 ml of 1CB at $OD_{550} = 2.0$ $(1.0 \times 10^9\,cfu\,ml^{-1})$ dosed at time zero and again after 3 h.

microbial degradation. The initial amount of TCE in each model aquifer was calculated by taking the average concentration adsorbed to three 1 g samples and multiplying it by the total amount of sediment. Final concentrations were measured at the anode, middle and cathode ends of the sediment bed by taking the average of three 1 g samples at each location. The total TCE remaining was then estimated by multiplying each of those averages by one-third the total weight of the sediment. In three experiments, where model aquifers were treated with *E. coli* W3110, or not amended with cells (controls), only 10–11% of the sorbed TCE was removed. In these same experiments, contaminated sand inoculated with two doses of 3×10^8 cells/dose of the TCE degrading strain *B. cepacia* 1CB showed a 72–89% decrease of the sorbed TCE (Fig. 5).

In addition to demonstrating degradation with model aquifer solids, we also contaminated actual aquifer solids with very high concentrations of TCE. This material, obtained from the EPA laboratory at Ada, Oklahoma, was a very fine grained ($<160\,\mu m$ average diameter) aquifer sediment which adsorbed approximately 300 ppm TCE. The model aquifer with strain 1CB had 69% or 207 ppm total TCE removed from the sediment, while a total of 38% of the sorbed TCE was removed from the control aquifer (Fig. 6).

4. Discussion

At high pH values most bacteria have a net negatively charged surface and at low pH a net positive charge due to a number of polymers which carry ionizable groups in the membrane. A net negative surface charge at pH 7.0 was verified in preliminary

experiments that determined unidirectional movement toward the anode for all of the bacterial strains tested. The composition of bacterial surface polymers is determined by genetics and growth conditions. Strain differences in net surface charge have been used to separate single strains from mixed populations [10]. Transport rates observed in these electrokinetics experiments were strain specific. The presence of two peaks of bacteria observed in the transport rate experiments with *E. coli* W3110 may represent a subpopulation with a different net surface charge. In general, the higher the pH, the greater the negative surface charge, which increases the electrophoretic mobility of the cell [25]. Of particular interest was the identical electrokinetic transport rates for the wild-type and adhesion-deficient G4. In transport experiments performed with hydraulic flow in the large model aquifer, the wild type was not detected at the downstream sampling points, whereas the adhesion mutant 1CB traveled at the same rate as the water flow ($5\,cm\,h^{-1}$ [26]). These observations suggest that the adhesion-deficient phenotype of the mutant is not due to a change in the surface charge of the organism because identical electrokinetic transport rates are due to identical surface charge density. In addition, these results suggest that electrokinetic transport may overcome most of the effects of bacterial adhesion on transport through aquifer sediments because the wild-type strain was not transported by hydraulic flow, but was electrokinetically transported.

The effect of pH on transport rate was tested within a range of pH values that are optimal for most bacteria. Values below pH 5.5 and above pH 8.5 are deleterious to the majority of bacterial strains. At pH 5.5, the negative charge on the cell may have been reduced to the point where the cells were moving very slowly in the electric current and therefore had not traveled 6 cm during the 6 h experiment. Alternatively, the low pH may have resulted in a net neutral or positive charge on the cells, causing them to stop or move in the opposite direction toward the cathode. Net positive surface charges have been measured in cells held at pH 2 [25]. In addition to increasing electrophoretic mobility, an increase in negative surface charge on the cell may increase the electrostatic repulsion between the cell and the surface of the aquifer solids, therefore decreasing adsorption and increasing the number of cells that travel the full length of the model aquifer, as seen in our pH experiments. Bacteria were sampled at only one point; therefore there was no attempt to determine percent recovery or percent survival in these experiments. The relative number of cells recovered in these experiments is probably indicative of changes in surface charge density at various pH. Overall, the results of the pH experiments suggest that the maximum electromigration rate obtainable would be strain specific and would be dictated by the highest pH at which the cells are still metabolically active.

The grain size distribution, which in turn determines the interstitial pore volume of the soil or sediment, is of particular importance in determining the rate and number of cells that can be transported by electrokinetics. The doubling of transport rates observed in the fine-grained play sand over the Ottawa sand were probably the result of the greater pore volume of the play sand ($0.31\,ml\,cm^{-3}$ for play sand vs $0.23\,ml\,cm^{-3}$ for Ottawa sand). Reduced rates of transport and reduced recovery of cells were observed in both the EPA aquifer solids and the two soils, indicating that the presence of silt and clay which reduces the pore size and volume will retard the bacteria. This technology is applicable to soils and sediments that have pore sizes sufficient to allow bacterial cells

to pass through them. Unlike other electroremediation technologies that take advantage of the fact that in dense clay soils electroosmotically driven water moves much faster and in greater quantities than hydraulically driven water, this application is not suitable for very dense, low conductivity soils and sediments. One potential application for this technology would be for remediating contaminated clay formations within a more permeable aquifer. Electroosmotic flow could be used to draw the contaminated water out of the clay, while the same electric field could be used to hold degradative bacteria at the clay/sediment interface to degrade the contaminants as they are drawn out of the clay.

The rate of bacterial transport measured at a voltage gradient of $4\,V\,cm^{-1}$ ($3.3\,cm\,h^{-1}$) in the large bench-scale aquifer was faster than that measured in the smaller model aquifers which were run at a voltage gradient of $10\,V\,cm^{-1}$ ($3\,cm\,h^{-1}$). Maximum bacterial electromigration rates of $1.44\,m\,day^{-1}$ obtained in this study are approximately 20 times the fastest rate measured for motile bacteria passing through unconsolidated sand cores [27]. Even faster rates may be obtained with strains tolerant of higher pH conditions or in aquifer solids with higher conductivity. In addition to enhancing the rate of movement, the electric field can also provide directionality to the bacteria being moved through the aquifer. Bacteria will move towards the positive electrode even if movement is tangential to the direction of the flow in the aquifer. In this way, transport of the bacteria is no longer passively dictated by the speed and direction of the groundwater flow. The apparent attenuation of cells in the large bench-scale aquifer, as evidenced by the decreasing recovery of cells with increasing distance from the injection point, may be due to dispersion of the cells with distance, causing them to move laterally with less recovery along the midline with increasing distance. Alternatively, this loss of cells may be due to bacteria being trapped in the sediment.

The results of electrokinetics experiments with TCE-contaminated sediment suggest that these bacteria can actively degrade while being transported in an electric field. Although not all of the TCE was removed in model aquifers with added ICB, a larger inoculum or a slower rate of transport could potentially result in greater degradative losses. For highly contaminated sediment, slower movement of the introduced strains, multiple injections, or a continuous injection over a longer period of time are strategies that could be effective in completely eliminating the source of the contamination in a groundwater aquifer: the organic contaminant sorbed to the solids. The fact that the electrokinetic transport of non-degradative bacteria through TCE-contaminated sand did not effect greater removal of TCE than the control suggests that the removal of TCE in the presence of *B. cepacia* 1CB was due to the degradative ability of the microorganisms and not simply sorption to the bacteria, although biosorption has been shown to increase contaminant transport in soil systems [28].

Electroosmotic water flow would not be expected to occur to a great extent in the experiments described due to the relatively high permeability of the matrix materials used and the ability of the water level to adjust freely. The fact that the TCE moved in the direction of the cathode does suggest either that there is some electroosmotic flow occurring or that the TCE is acting as a polar compound and has some positive charge associated with it. Although the potential for inducing a hydraulic gradient exists in these model aquifers, the hydraulic gradient would have been opposite to that of the

observed direction of bacterial transport and would not have increased the rates of bacterial transport observed.

Desorption of TCE from aquifer solids and its transport toward the cathode is an additional benefit of electrokinetics. This process brings the contaminant closer to the well where the bacteria are being injected, decreasing the distance that the bacteria need to be transported to contact the contaminant. Future work in this area will involve establishing the kinetics of degradation under electrokinetic conditions. These studies will determine the density and residence time of bacteria in an aquifer necessary to effect a complete cleanup. It is expected that the degradation kinetics will depend upon the concentration of the contaminant, the type of aquifer solids, and the rate at which the bacteria are traveling through the aquifer.

The use of electrokinetics for transporting degradative strains for bioremediation is applicable to any organic contaminant that adsorbs to aquifer solids. These include the non-aqueous phase liquids (NAPLs) and other contaminants that respond poorly to attempts to remediate by pump-and-treat technologies.

Acknowledgements

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