Energy Sources, 25:931–943, 2003
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ISSN: 0090-8312 print/1521-0510 online
DOI: 10.1080/00908310390221318

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Nutrient Amendment for the Bioremediation of a Chromium-Contaminated Soil by Electrokinetics

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This article presents the results of a preliminary laboratory investigation wherein electrokinetics was used for the delivery of nutrients to metal-reducing micro-organisms in a low permeability clayey soil. In particular, the micro-organisms were used to reduce a toxic and mobile heavy metal (hexavalent chromium or Cr(VI)) to a less toxic and immobile form (trivalent chromium or Cr(III)). Three bench-scale electrokinetic experiments were conducted using kaolin as a model low permeability clayey soil, and the kaolin was artificially contaminated with Cr(VI) at an initial target concentration of 1,000 mg/kg. All the experiments were conducted with a constant electrical voltage gradient of 1.0 V/cm and included a control test without micro-organisms or nutrients, a test with micro-organisms but without nutrients, and a test with micro-organisms and supplemental nutrients, specifically acetate, phosphate, and ammonium. The results showed that acetate and phosphate amendment by electrokinetics was effective because both nutrients electromigrated into the soil. Moreover, the results indicate that employing the micro-organism cultures improved Cr(VI) reduction. These results suggest that nutrient amendment by electrokinetics for the bioremediation of heavy metals has great potential; however, the microbial strains responsible for Cr(VI) reduction must be identified so the electrokinetic system can be engineered to provide the optimal nutrient, pH, and environmental conditions for these strains.

Keywords bioremediation, chromium, electrokinetics, immobilization, pollution, soil

Heavy metal pollutants adversely affect public health and the environment, and the costs associated with the remediation of heavy metal-contaminated sites can be substantial. One of the most toxic and common heavy metal contaminants is hexavalent chromium

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Received 29 June 2002; accepted 9 August 2002.

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(Cr(VI)) (Gotlieb et al., 1991; James, 1996). The higher toxicity and mobility of Cr(VI) compared to trivalent chromium (Cr(III)) causes it to be of greater concern (Ross et al., 1981).

Bioremediation is a low cost treatment technology that employs either indigenous, naturally occurring, or bioaugmented micro-organisms to reduce, remove, and/or immobilize contaminants (McCullough et al., 1999). Certain micro-organisms have the unique ability to adapt to environments with elevated metal concentrations, and different strains have been evaluated for reducing Cr(VI) to Cr(III) (Fude et al., 1994; Lovely and Phillips, 1994; Cher and Hao, 1996). To date, most of the heavy metal bioremediation research has been focused on aqueous-based contaminants using pure cultures such as *Pseudomonas putida* (Ashi et al., 1990), *desulfovibrio vulgaris* (Lovely and Phillips, 1994), and *Escherichia coli* (Shen and Wang, 1994). These and other studies have indicated that anaerobic bacteria that excrete chromate reductase were very effective at reducing Cr(VI) concentrations.

Bioremediation shows great promise for Cr(VI) reduction, but it may be limited in clayey soils due to their low hydraulic conductivity, which makes it nearly impossible to introduce nutrients necessary for microbial metabolism. However, the electrokinetically induced transport mechanisms of electromigration and electro-osmosis may facilitate nutrient introduction for micro-organisms located in low permeability soils. Electrokinetics essentially involves installing wells/drains, inserting electrodes, and applying a low electric potential across the electrodes. Nutrients may be added to the solution at the anode and/or cathode electrode, and, as a result of electromigration and/or electro-osmosis, the nutrients are transported from the electrodes into the soil. Basically, electromigration refers to the transport of charged species toward the electrodes, whereas electro-osmosis refers to the bulk movement of liquid toward the electrodes (generally from the anode to the cathode; Eykholt, 1992; Acar and Alshawabkeh, 1993).

This article presents the results of a preliminary study that was conducted to evaluate electrokinetics for delivering nutrients to micro-organisms to biostimulate heavy metal reduction in a low permeability soil.

Experimental Procedure

Materials

Kaolin was used as the model low permeability soil for this study. It is a clay soil that consists mostly of the mineral kaolinite, and it is virtually free of organic matter. Kaolin has a cation exchange capacity of 1.6 meq/100 g and a low acid buffering capacity. The hydraulic conductivity is of the order of 10^{-8} cm/s, which makes hydraulic flow-controlled remediation techniques ineffective and/or expensive with this soil.

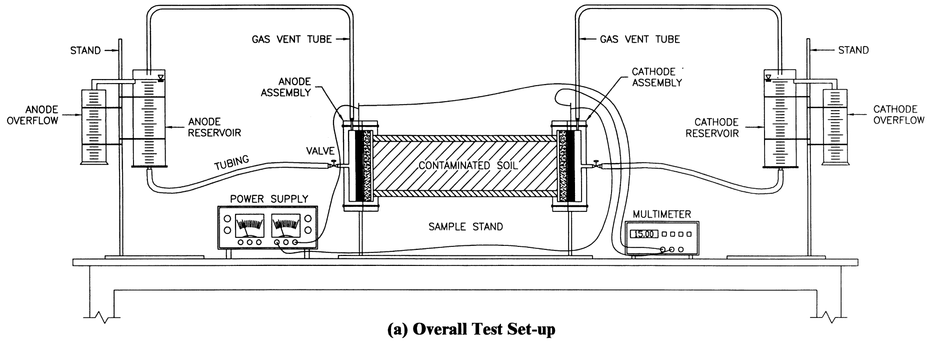
The anaerobic sludge for this study was obtained from the Stickney Water Reclamation District of Chicago. The sludge was maintained by adding 4 g/L of sodium acetate (CH_3COONa, as a primary carbon source) and 3 g/L of potassium nitrate (KNO_3) and ammonium acetate (CH_3COONH_4) as nitrogen sources. Approximately 10% of the total nitrogen was added as ammonia to account for the sludge fraction that cannot immediately utilize nitrogen in the nitrate form. After the nutrient amendments, the initial pH, redox, and temperature of the sludge were determined to be 7.13, 120 mV, and 23°C, respectively.

The sludge was acclimated to elevated Cr(VI) concentrations following the procedure's successful implementation for the preliminary aqueous phase batch experiments (data not shown). The acclimation process was conducted in a 1 gallon, airtight glass bottle. The sludge was amended with 79 mg/L, 158 mg/L, and 237 mg/L of Cr(VI) on day 1, 8, and 10, respectively. The sludge was then maintained with the supplemental nutrients and 237 mg/L Cr(VI) amendments until the initiation of the experiment. After adding the Cr(VI), the pH, redox, and temperature of the sludge were determined to be 9.9, −30 mV, and 22°C, respectively.

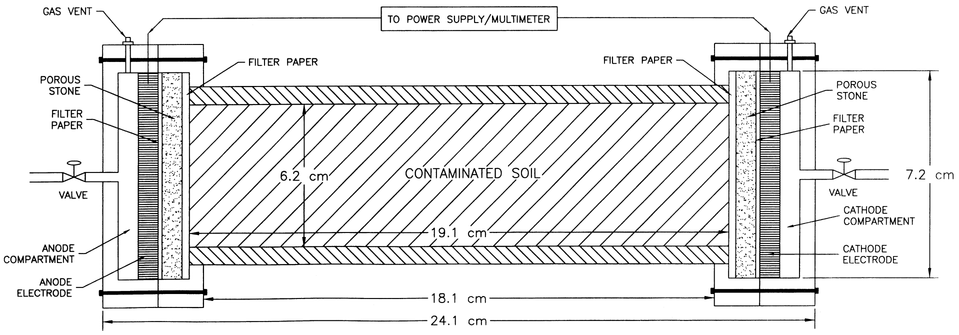
Electrokinetic Testing Procedure

For each test, approximately 1,100 g of dry soil was contaminated with chromium in the form of potassium chromate (K_2CrO_4). To simulate typical contaminated site conditions, the Cr(VI) concentration was targeted for 1,000 mg/kg and the moisture content was targeted for 35%. The amount of deionized water to yield these conditions (about 385 ml) was measured and placed in a beaker. Then the K_2CrO_4 needed to yield a 1,000 mg/kg Cr(VI) concentration in the dry soil was dissolved in the deionized water and subsequently mixed with the soil.

The contaminated soil was placed in the electrokinetic reactor in layers with uniform compaction and then allowed to equilibrate for 24 h (Figure 1). The reactor consisted of a cell, 2 electrode compartments, 2 reservoirs, and a power source (Reddy et al., 1997; Reddy and Parupudi, 1997). Potable water or nutrient solution was used in the electrode reservoirs. Potable water was used to simulate the water supplies available at contaminated sites. A constant voltage gradient of 1.0 V/cm was applied across the soil speci-



(a) Overall Test Set-up



(b) Cell Details

Figure 1. Schematic of electrokinetic reactor.

men. The electrical current, electro-osmotic flow, as well as the pH, redox, and electrical conductivity (EC) of the electrode solution were measured at specified intervals during the experiment. Each test was continued until the current stabilized and/or the electro-osmotic flow became negligible. At the conclusion of testing, the soil was extruded from the test cell, sectioned into 5 parts, and weighed. The pH, redox potential, and EC of the soil sections were measured. Soil samples from each section were acid digested using the USEPA 3050 procedure (USEPA, 1986) to determine the total chromium (Cr(total)) present. In addition, Cr(VI) concentrations were determined by alkaline digestion via USEPA 3060A. The solutions resulting from the digestion procedures were analyzed using an atomic absorption spectrophotometer (AAS) to determine the Cr(total) and Cr(VI) concentrations. Cr(III) values were ascertained by performing a mass balance (Cr(III) = Cr(total) − Cr(VI)). Duplicate soil and solution samples were tested during the acid and alkaline digestion procedures to ensure the accuracy and reproducibility of the test results.

Nutrient migration was evaluated by analyzing each soil section obtained after the electrokinetic treatment was concluded. For this analysis, a sample of wet soil weighing 10 g was first suspended in 20 mL of deionized water. The suspension was then agitated on a shaker table for 1 h at 1,000–2,000 rpm, and the solids were subsequently removed via centrifugation. The supernatant was then analyzed by ion chromatography to determine the acetate and phosphate concentrations. These concentrations were then used to calculate the acetate and phosphate concentrations in the soil. During the ion chromatography analysis, duplicate soil samples were tested to ensure the accuracy and reproducibility of the test results.

A total of 3 laboratory electrokinetic experiments were conducted in this study. The first test (EK-CONTROL) was conducted without micro-organisms or nutrients to serve as a baseline test for comparison purposes. In the second test (EK-BIO-1), an anaerobic culture of micro-organisms was acclimated to Cr(VI) and mixed with the Cr(VI)-contaminated soil, but nutrient delivery was not provided. In the third test (EK-BIO-2), the same acclimated, anaerobic culture was mixed with the Cr(VI)-contaminated soil, only in this test, nutrient delivery was provided during electrokinetics. In particular, sodium acetate (CH_3COONa) and sodium phosphate (NaH_2PO_4) were introduced at the cathode and ammonium chloride (NH_4Cl) was introduced at the anode. Carbon, nitrogen, and phosphorus are the primary nutrients required for microbial metabolic processes (McCullough et al., 1999). The test results were analyzed to evaluate the migration of Cr(VI), the extent of nutrient delivery, and the reduction of Cr(VI) to Cr(III) that occurred due to the presence of the anaerobic culture.

Results

Table 1 summarizes the soil conditions that existed before electrokinetic treatment. This table indicates that adding the microbial culture decreased the pH of the soil slightly from 7.63 in the baseline test to 7.56 or 7.46 in the tests employing micro-organisms. Furthermore, after the microbial culture was added, the redox potential and EC increased from 95 mV to about 160 mV and from 2.01 to >200 μS/cm, respectively.

The redox potential is indicative of the type of microbial respiration, aerobic or anaerobic, that is occurring, and redox readings above 25 mV are characteristic of an aerobic environment (Bishop and Tong, 1999). At a lower redox range of -100 mV to 25 mV, facultative micro-organisms may thrive. Facultative micro-organisms may be capable of switching their respiration from anaerobic to aerobic (or vice versa) by using an alternative terminal electron acceptor (other than O_2) that is available. When the redox

Table 1
Soil conditions prior to electrokinetic treatment

Test	pH	Redox potential (mV)	Electrical conductivity (μS/cm)	Total Cr (mg/kg)	Cr(VI) (mg/kg)	Cr(III) (mg/kg)
EK-CONTROL	7.63	95	2.01	895.09	810.65	84.44
EK-BIO-1	7.56	165	203	1064.84	953.17	111.67
EK-BIO-2	7.46	160	221	1066.96	988.22	78.74

potential reaches an even lower range of −100 mV to −200 mV, the environment is considered to be anaerobic, and readings below −200 mV indicate an obligate (strictly) anaerobic environment (Bregnard et al., 1996; Norris, 1994). As seen in Table 1, prior to electrokinetic treatment, the soil redox measurements for the 3 electrokinetic experiments suggest that an aerobic soil environment existed. Thus it appears that the experiments employing the anaerobic cultures had a minimal amount of microbial activity. It is important to note, however, that when the micro-organisms were being acclimated to Cr(VI), the culture possessed a redox potential of −30 mV, which is typically in the facultative range. Consequently, prior to electrokinetic treatment, the facultative micro-organisms may have used aerobic respiration and continued to be active.

Table 1 shows that, compared to the baseline test, the initial total chromium concentration was higher in the tests employing the micro-organisms. It should be noted that prior to introduction to the soil, the micro-organisms were acclimated with Cr(VI), so it seems that some of the residual chromium from the acclimation process accompanied the culture when it was added, and this increased the initial chromium concentration in the soil. In addition, Table 1 shows that around 10% of the initial chromium spiked into the soil for the baseline experiment was in the form of Cr(III), and further analysis will be needed to ascertain the reason for this initial reduction of Cr(VI). For the tests that employed the micro-organisms, the approximate 10% reduction of Cr(VI) to Cr(III) may be partially explained by the presence of the micro-organisms, which may have generated microbial enzymes during the acclimation phase that contributed to Cr(VI) reduction.

Current

The current passing through the soil depends on the soil conductivity, which largely depends on the concentration and type of ionic species that are present in the pore fluid. Generally, if the ionic concentration is high, then the current will be high. Figure 2a shows that the current in all 3 tests quickly reached a peak value of around 70 mA within about 20 h, then the current decreased substantially over the next 100–300 h and finally stabilized.

At the start of testing, the current is comparatively low because the solution within the electrode compartments has a low ionic concentration. However, within a short amount of time, any salts present in the soil dissolve and ions begin electromigrating toward the electrodes. As a consequence, there is often a rapid increase in the conductivity and current through the soil at or near the start of testing. Over time, the ion electromigration and current decrease as the free cations and anions reach their respective electrode destination and become neutralized (Eykholt, 1992). In addition, the products from the

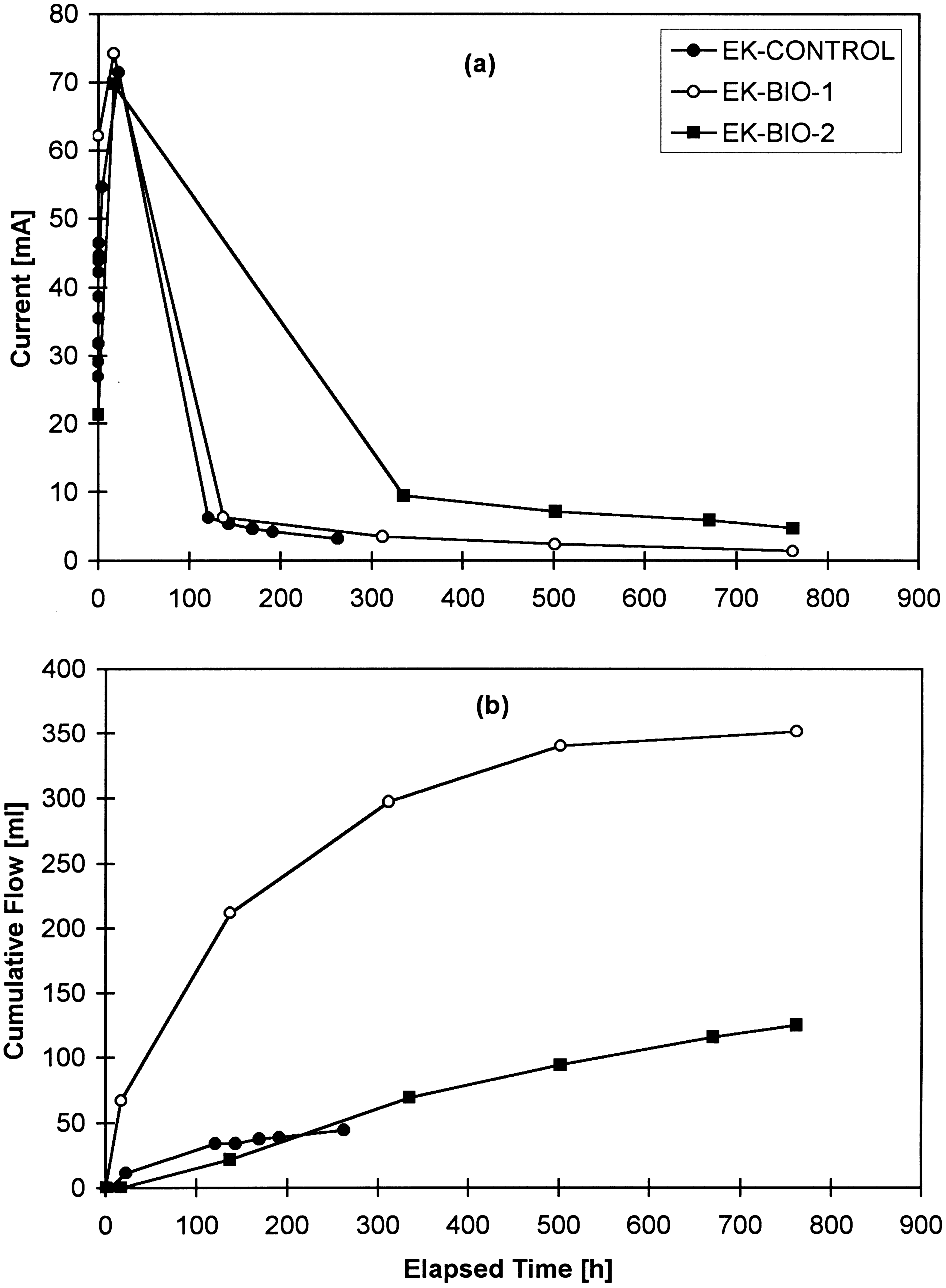


Figure 2. (a) Electric current and (b) cumulative electro-osmotic flow during electrokinetic treatment.

electrolysis reactions (H^+ and OH^- at the anode and cathode, respectively) increase with time, and these products may contribute to neutralizing oppositely charged species, or they could electromigrate toward the oppositely charged electrode. Moreover, the changes in pH due to these electrolysis products affect the solubility of other charged species, and this could affect the current. In the EK-BIO-2 test, the current remained slightly higher and persisted slightly longer than the current in the EK-CONTROL and EK-BIO-1 tests, and this was attributed to the introduction of nutrients to the system (Figure 2a).

Electro-Osmotic Flow

For all 3 experiments, the electro-osmotic flow was directed from the anode toward the cathode, so the volume in the anode reservoir decreased while the volume in the cathode reservoir increased. The electro-osmotic flow is primarily generated by electromigration, so, usually, a high flow occurs near the initial stages of testing when the current is high. Compared to the EK-CONTROL test, the high flow rate in the EK-BIO-1 test was attributed to the ions added along with the micro-organisms. The flow in the EK-BIO-2 test was lower than EK-BIO-1, possibly due to the introduction of the nutrients. By introducing a greater number of anionic species, such as acetate and phosphate, at the cathode, there was a greater amount of electromigration occurring toward the anode (opposing the direction of electro-osmotic flow). It appears that this electromigration of anions toward the anode partially counteracted the electromigration of cations toward the cathode, and this resulted in less flow at the cathode. Apparently, for EK-BIO-2, the greater number of ionic species being introduced as well as the conflicting directions of electromigration contributed to reducing the initial flow rate toward the cathode as well as generating a low and sustained flow rate toward the cathode.

pH and Redox Potential

Table 2 shows the conditions at the electrodes at the end of the electrokinetic treatment. A comparison of values in Table 1 and Table 2 reveals that the solution chemistry at the electrodes changed considerably as a result of the electric potential application and the electrolysis reactions.

As seen in Figure 3a, all the tests had a pH through the soil that reduced to low, acidic, values. The only exception occurred in the EK-CONTROL test, where the soil section adjacent to the cathode had a pH of about 12. The other 2 tests, which were conducted with micro-organisms in the soil, had low pH values that extended completely across the soil profile. These results indicate that the acidic front generated by the electrolysis reaction at the anode was transported by electromigration and electro-osmosis toward the cathode. In the control test, however, it seems that the acid front did not flush completely

Table 2
Conditions at electrodes at the end of electrokinetic treatment

	Anode compartment and reservoir			Cathode compartment and reservoir		
Test	pH	Redox potential (mV)	Electrical conductivity (μS/cm)	pH	Redox potential (mV)	Electrical conductivity (μS/cm)
EK-CONTROL	2.18	376	3.25	13.07	−190	12.27
EK-BIO-1	2.63	476	NA	10.67	−34	NA
EK-BIO-2	2.75	468	NA	11.12	−63	NA

NA = not available.

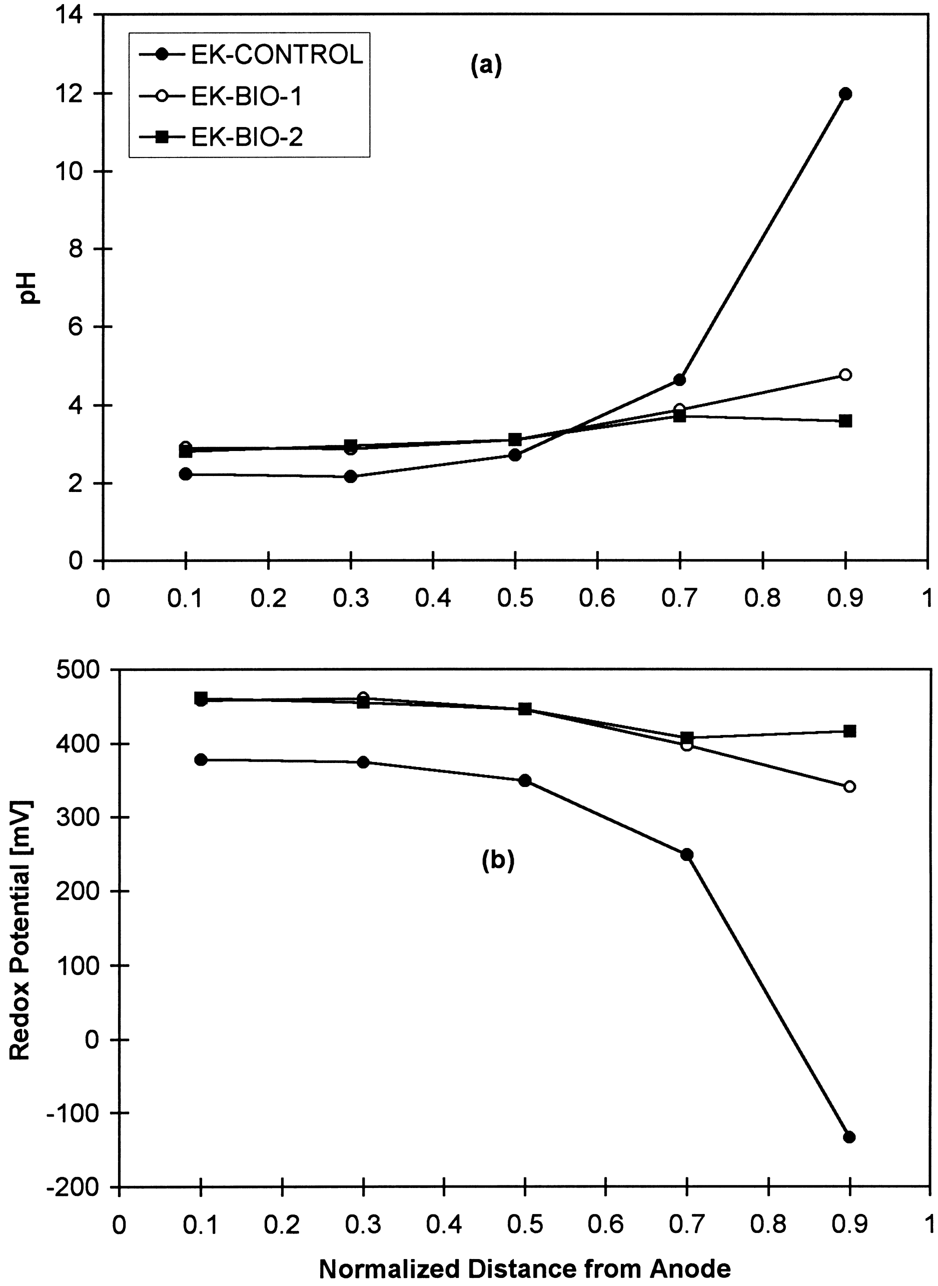


Figure 3. (a) pH and (b) redox potential of soil after electrokinetic treatment.

across the soil, and there was some transport of hydroxyl ions into the soil from the cathode electrolysis reaction. Overall, this trend, where a low pH exists throughout most of the soil, is representative of pH profiles in kaolin after undergoing electrokinetic treatment (Reddy et al., 1997; Reddy and Parupudi, 1997).

Several researchers have found that when environmental conditions change, some bacteria may self-regulate the surrounding soil pH by releasing exudates to return the soil to a more optimal pH environment (pH 5.5–7.5) (Cookson, 1995; McKane and Kandel, 1996). By observing Figure 3a and comparing the EK-CONTROL test to the tests employing micro-organisms (EK-BIO-1 and EK-BIO-2), it can be seen that the tests that used micro-organisms had slightly higher pH values across the first 3 soil sections nearest the anode and had lower pH values near the 2 sections closest to the cathode. This suggests that the micro-organism activity might have contributed to changing the pH, but the pH was primarily controlled by the electrolysis reactions.

Figure 3b shows that all 3 tests had oxidizing conditions across the first 4 sections nearest the anode, and only one test (EK-CONTROL) possessed reducing conditions, which occurred in the soil section adjacent to the cathode. Initially, researchers believed that only sulfate-reducing bacteria in a reducing (−220 mV) environment were capable of converting Cr(VI) to Cr(III), but recent studies have documented that facultative species, which can survive in an aerobic environment, may also convert Cr(VI) to Cr(III) (Dmitrenko et al., 1997; Wakatsuki, 1995).

Nutrient Migration

Since acetate and phosphate exist as anionic species, they were expected to electromigrate toward the anode, whereas ammonium exists as a cationic species, so it was expected to electromigrate toward the cathode. Figure 4 shows that the acetate concentration ranged from nearly 10,000 mg/kg in the soil region adjacent to the cathode to 10 mg/kg in the soil region near the anode, and this indicates that acetate anions were transported from the cathode toward the anode. Furthermore, Figure 4 shows that a high concentration of phosphate existed near the center of the soil specimen, which suggests that phosphate anions were also electromigrating toward the anode. The 2 soil regions nearest the cathode were deficient in phosphate, which suggests that nearly all the phosphate mass had been

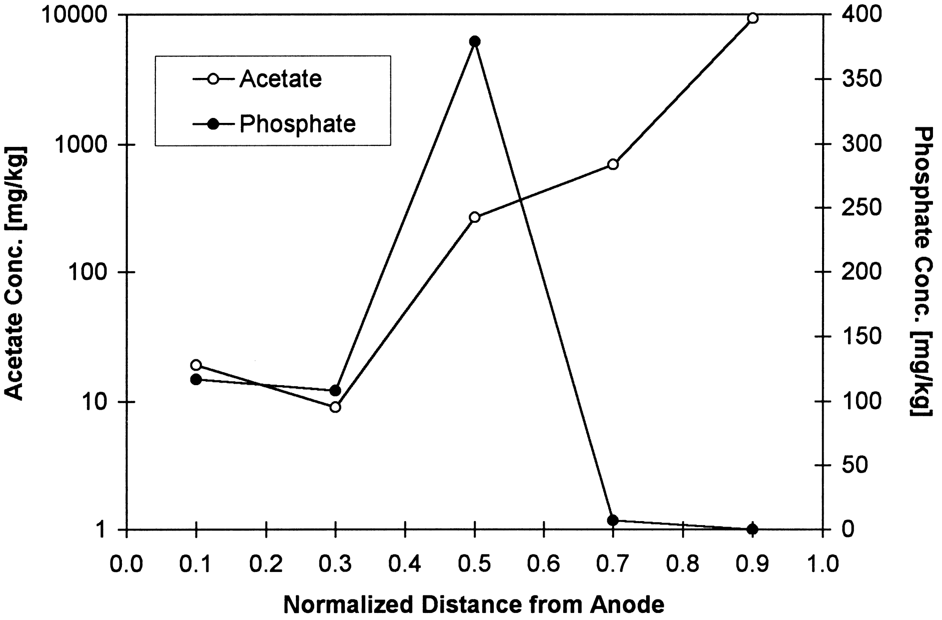


Figure 4. Acetate and phosphate concentrations in the soil after electrokinetic treatment.

transported by electromigration from the cathode reservoir and the soil region nearest the cathode to the center of the soil specimen.

Calculations revealed that a small amount, approximately 2% (985 of 52,040 mg) of the total initial mass of acetate introduced into the cathode reservoir, was transported into the soil. Therefore an ample source of acetate remained in the cathode reservoir, and, if the test duration was increased, the acetate concentration may have increased throughout the soil specimen. Similar calculations also revealed that 99 mg of phosphate had been transported into the soil, which is 90% (99 of 110 mg) of the total initial mass that was introduced into the cathode reservoir. This reinforces the hypothesis mentioned earlier that the majority of the phosphate mass was transported to the center of the soil sample by electromigration from the cathode reservoir and soil region nearest the cathode.

It is important to note that the micro-organisms may have affected the concentrations of acetate and/or phosphate in the soil, but overall the results of this study corroborate the findings of other investigations that have found substantial amounts of nutrients could be amended into low permeability soils using electrokinetics (Elektorowicz and Boeva, 1996; Gent, 1998; Parker et al., 2000). Since the electro-osmotic flow was directed from the anode toward the cathode, and the nutrient anions were transported from the cathode toward the anode, it is evident that the dominant process for nutrient transport is electromigration.

Chromium Migration and Biological Reduction

Generally, under neutral or high pH conditions, Cr(VI) exists as a soluble and mobile chromate (CrO_4^{2-}) anion, but, under low pH conditions, it commonly changes to the bichromate ($HCrO_4^-$) anion, which is more susceptible to adsorption (Hicks and Tondorf, 1994; Reddy and Parupudi, 1997; McCullough et al., 1999). Thus the high pH and reducing conditions that develop as a result of the electrolysis reaction at the cathode are favorable for Cr(VI) solubility and electromigration; conversely, the low pH and oxidizing conditions that result from the electrolysis reaction at the anode tend to cause Cr(VI) adsorption, precipitation, or reduction.

Figures 5a–c show the Cr(total), Cr(VI), and Cr(III) distributions in the soil, respectively, for all the tests at the end of electrokinetic treatment. The results of the EK-CONTROL test were consistent with the results from previous studies on the electrokinetic remediation of Cr(VI) (Reddy and Parupudi, 1997; Reddy et al., 1997; Chinthamreddy, 1999). Figure 5a indicates that in all the tests chromium electromigrated toward the anode, however, only a small amount of chromium was detected in the anode reservoir. It can be seen that for all 3 experiments, after treatment, the majority of the chromium resided in the 2 sections nearest the anode. Conversely, due to the higher mobility of chromium under high pH and/or reducing conditions, the amount of chromium measured in the cathode reservoir solution and in the 2 sections nearest the cathode was comparatively negligible. An assessment of the electrokinetic process was performed using geochemical modeling (MINEQL$^+$), and this model showed a high accumulation of Cr(VI) in the sections closest to the anode due to the adsorption of Cr(VI) to the clay particle surfaces under low pH conditions (Reddy et al., 2001).

As discussed above, the chromium electromigration in the EK-CONTROL test was similar to the tests that employed the micro-organisms (EK-BIO-1 and EK-BIO-2), but although the 3 tests had similarities, there were also some important differences. As seen in Figures 5a and b, the chromium concentration steadily increases toward the anode in the tests that used the micro-organisms, but in the EK-CONTROL test, the

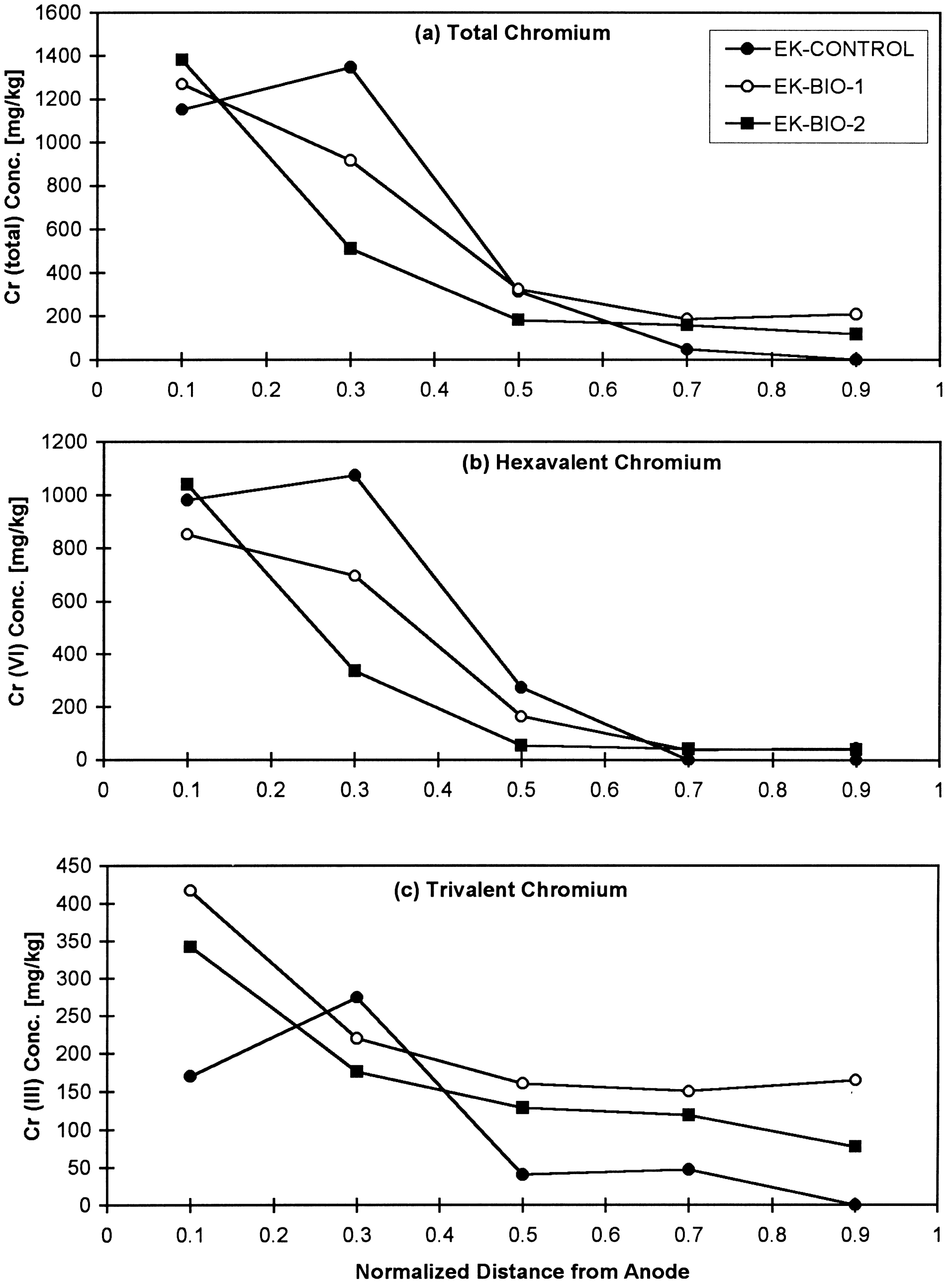


Figure 5. (a) Total Cr, (b) Cr(VI), and (c) Cr(III) soil concentrations after electrokinetic treatment.

concentration in the second soil section from the anode had a higher concentration than the soil section directly adjacent to the anode. In addition, Figure 5c shows that compared to the EK-CONTROL test, a large amount of Cr(VI) was reduced to Cr(III) near the anode section in the tests using micro-organisms, and, except for the second section from the anode, the Cr(III) concentration was greater in the tests using micro-organisms. These differences, especially the greater Cr(III) concentration, strongly suggest that the extracellular enzymes and exudates produced by the bacterial culture were somewhat successful at chromium reduction, and they were not completely inhibited by the factors that accompanied the electrical potential application. Nevertheless, as shown in Figure 5b, high Cr(VI) concentrations were measured in all the tests near the anode, so, evidently, the microbial reduction of Cr(VI) to Cr(III) was severely limited. Furthermore, a comparison of the EK-BIO-1 and EK-BIO-2 tests in Figure 5c shows that the Cr(III) concentration was generally greater in the EK-BIO-1 test, where supplemental nutrients were absent, so it appears that the nutritional biostimulation that was provided for increasing Cr(VI) reduction was ineffective.

Conclusions

The micro-organism activity under the electrokinetic conditions used in this study was not conducive to reducing Cr(VI) to Cr(III), with or without nutritional supplements. Many interrelated factors may have been responsible for these results, such as the high toxicity of the initial Cr(VI) concentration to the microbes, the low pH environment in the soil produced by the electrolysis reaction at the anode, the effect of the electric potential on the micro-organisms, and/or the competition between different ionic species. Although one or more of these factors may have hindered a Cr(VI) reduction, a minor amount of microbial reduction of Cr(VI) was evidenced by the larger Cr(III) concentrations that existed in the tests using micro-organisms.

Although the electrokinetic test conditions were not conducive for the microbial reduction of Cr(VI), this study demonstrated, and reinforced the findings of other studies, that nutrients can be delivered into a low permeability soil, such as kaolin, via electrokinetics. For this investigation, the results showed that a substantial amount of acetate and phosphate electromigrated into the soil from the cathode reservoir. This is an important finding because alternative methods for nutrient delivery into low permeable soils are often based on hydraulic flow, and these hydraulic techniques are commonly ineffective or cost prohibitive. This preliminary study was also important because it shows that the electrokinetic treatment process needs to be engineered to sustain microbial activity. Although some microbes may have the capability to buffer or control their pH environment, it was evident from these experiments that the environmental conditions generated by electrokinetics are too extreme for microbial survival. Furthermore, the results suggest that the micro-organism activity was not limited by lack of nutrients. Therefore additional research is warranted to engineer the electrokinetic process and optimize the nutrient, pH, and environmental soil conditions for increasing microbial activity.

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