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Coupled electrokinetic and biological remediation method leads to improved treatment of chlorinated solvents at high sulfate, transport limited sites

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Chlorinated solvents are some of the most pervasive pollutants found in groundwater and drinking water sources in the United States (U.S.). In the early 2000s, bioremediation emerged as a novel and effective technology, but was limited by challenges to delivery and transport of nutrients and microbes. Electrokinetic bioremediation (EK-Bio) has since emerged as a promising alternative to solve these limitations, delivering successful results at the lab and pilot scale. EK-Bio can be applied at sites where traditional bioaugmentation, the transformation of pollutants via an added microbial culture, is transport limited. The application of direct current *in situ* in electrokinetic (EK) remediation facilitates transport of the microbial culture and substrate in the subsurface. Despite this recent surge in interest surrounding EK-Bio, it is not clear how this technology would perform at a site with elevated levels of alternative electron acceptors, another common barrier to successful bioremediation. Our objectives were to use bench scale reactors to 1) determine which reactions and processes would dominate when using EK-BIO to treat TCE contamination at a site with high levels of the alternative electron acceptor sulfate, 2) compare EK-Bio to a traditional bioremediation application without electrokinetics, and 3) understand the effect of EK-Bio on the microbial community under these conditions. Our results showed complete transformation of TCE to ethene and acetylene by EK-Bio, while only 15% of TCE was transformed to cis-DCE and VC via traditional bioaugmentation. Instead, the majority of the TCE was converted to acetylene, likely due to its electrochemical reduction at the cathode. EK-Bio out performed traditional methods as it facilitated TCE biotic and abiotic transformation. Next generation sequencing analysis showed the microbial community in the EK-Bio reactor was highly enriched by the bioaugmentation culture, and community structure and diversity were minimally affected by the electrokinetic application. These results demonstrate that EK-Bio is an effective and promising remedy for treating chlorinated solvent contamination at transport limited sites with high concentrations of competing electron acceptors. This combined treatment strategy can be used to extend traditional bioaugmentation to a greater number of polluted sites, restoring more contaminated water systems for beneficial use.

Water Impact Statement

Trichloroethene (TCE) is one of the most widespread contaminants in groundwater affecting an estimated 4.5-18% of drinking water sources in the United States. Combined remediation technologies are required to address the increasingly complex sites which remain polluted. Here, we present a combined bioelectrochemical approach which improves treatment outcomes and extends applications of traditional technologies.

1. Introduction

Chlorinated solvents like perchloroethylene (PCE) and trichloroethylene (TCE) are common groundwater contaminants throughout the United States (U.S.) which cause concern due to their toxic properties and widespread occurrence [1,2]. Previously used as dry cleaning and degreasing agents, these chemicals entered the watershed due to accidental spills and improper disposal [3,4]. PCE has been detected in 4% of aquifers tested by the U.S. Geological Survey (USGS), and TCE has been measured in 4.5-18% of the country's drinking water supply sources[5,6]. Health issues associated with PCE and TCE range from damage to the nervous system, liver, kidney, and reproductive systems, to developmental issues, and possibly cancer[5,6]. PCE and TCE daughter product vinyl chloride (VC) is a known carcinogen[7]. Given the health effects associated with these compounds and their daughter products, complete removal or transformation to non-toxic ethene is required to protect human health[8].

One commonly used method for treating chlorinated solvent contamination is bioaugmentation, the *in situ* addition of a bacterial culture capable of dechlorinating PCE and TCE to ethene[9,10]. The key bacteria, *Dehalococcoides*, removes one chlorine atom at time and replaces them with hydrogen in a process known as microbial reductive dechlorination, transforming PCE to TCE, TCE to cis-dichlorethene (cis-DCE), cis-DCE to VC, and VC to ethene[11]. *Dehalococcoides*, are strict anaerobes that use H_2 as an electron donor and acetate as a carbon source [9]. They require moderate temperatures (25-40°C) and neutral pH conditions[11]. In the subsurface, H_2 and acetate can be delivered to *Dehalococcoides* through anaerobic fermentation of substrates like lactate[12]. Bioaugmentation using cultures with *Dehalococcoides* was developed as a treatment strategy in the 1990's, and hundreds of sites have since been successfully treated with this remedy[13]. Despite this success, there remain challenges to bioaugmentation efficacy. Two of the most substantial challenges to anaerobic bioremediation of chlorinated solvents are microbial competition from native soil bacteria and transport of bioaugmentation cultures and substrates *in situ* [14].

One of these challenges, transport limitations, can be addressed by pairing traditional bioaugmentation with technologies that improve delivery of nutrients and microbes, like electrokinetics (EK) [15]. EK is the application of direct current to the subsurface to induce transport *in situ*. Soluble molecules may be transported via movement of fluid through pore spaces (electroosmosis) and ions or other charged molecules may move to the oppositely charged electrode (electromigration and electrophoresis) [15]. When EK is

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combined with bioaugmentation (EK-Bio), the bioaugmentation culture and electron donor are added to the subsurface via traditional injection wells and transported via electrokinetic mechanisms in addition to the natural advective gradient[15]. It is important to note that the application of current causes electrochemical reactions at each electrode, namely production of oxygen gas at the anode and hydrogen gas, H_2, at the cathode according to the reactions below [16].

$$Anode - \ H_2O \rightarrow 2H^+ + \frac{1}{2}O_2 + 2e^- \ \#(1)$$

$$Cathode - \ 2H_2O + 2e^- \rightarrow 2OH^- + H_2 \ \#(2)$$

Further, these reactions generate a pH gradient with acidic conditions at the anode and basic conditions at the cathode[16]. Both the extreme pH fronts and the oxygen produced by the anode must be carefully managed at sites where microbial reductive dechlorination is employed to maintain the specific conditions required by *Dehalococcoides*.

Microbial competition for H_2 in the subsurface between *Dehalococcoides* and native soil bacteria is caused by high concentrations of alternative electron acceptors, like sulfate. High levels of sulfate are often found in PCE and TCE plumes due to natural sources, like atmospheric deposition, sulfate mineral dissolution, and sulfide mineral oxidation, or anthropogenic sources, like coal mines, power plants, and refineries [17,18]. Like microbial reductive dechlorination, microbial sulfate reduction is carried out with H_2 as an electron donor, leading to competition between sulfate reducing bacteria (SRB) and dechlorinating bacteria for the limited H_2 available *in situ* [17]. Further, sulfide inhibition of dechlorination also occurs at high concentrations (greater than 5mM) due to the toxicity of the sulfide species (H_2S and HS^-) produced from sulfate reduction [17]. These inhibitory effects are further exacerbated in the field when sites are flooded with electron donor as toxic sulfide species accumulate [17]. It is not clear how EK-Bio would perform at a site with elevated levels of alternative electron acceptors. Electrokinetic bioaugmentation of chlorinated solvents at sites with high concentrations of sulfate has not been extensively studied. It is possible that EK transport of substrate could favor SRB who can out-compete *Dehalococcoides* for H_2[19]. This scenario could cause a stall of microbial reductive dechlorination or generation of a reactive metal sulfide species capable of abiotic dechlorination[20–22]. A mixture of biotic and abiotic reactions could occur, including some electrochemical transformations of TCE which have been reported in closed recirculation systems with Pt, Pd, iron, and graphite electrodes[23–25]. Our objectives were to 1) determine which reactions and processes would dominate at a TCE contaminated site with high levels of the alternative electron acceptor sulfate, 2) compare EK-Bio to a traditional bioremediation application without electrokinetics, and 3) understand the effect of EK-Bio on the microbial community under these conditions.

2. Materials and methods

2. 1 Reactor Design and Set-up This experiment featured a combined electrokinetic bioaugmentation (EK-Bio) reactor and a traditional bioaugmentation reactor (Bio). The Bio reactor was operated with traditional bioaugmentation methods where diffusion is the main processes for mass transfer. No current was applied to the Bio reactor. The EK-Bio reactor was operated with a combined electrokinetic and bioaugmentation approach. Direct current was delivered to the EK-Bio reactor via power supply (Rigol DP832). Each of the two reactors was constructed from acrylic, consisting of a central soil compartment (40 cm long, 8 cm wide, 20 cm high; 6.4 L) between two electrode chambers (10 cm long, 8 cm wide, 20 cm high; 1.6 L). The soil and electrode compartments were divided with a plastic porous separator (Midland Scientific Inc, HDPE, 1.6mm, medium grade porosity). A grid of nylon Swagelok sampling ports fitted with rubber septa covered the top and front face of each soil chamber. Porous metal tubes made of rolled screening (nickel 200, wire mesh 70 x 70) were inserted into all the ports to allow collection of porewater for sampling. Graphite electrodes (Fine extruded rod, 1.27 cm OD, Graphite Store) were used for both the anode and cathode. Once the electrodes were in place, the electrode chambers were filled with glass beads (11mm OD) to decrease the electrode compartment volumes to approximately 280 mL. A gas bag (5L, PVF Tedlar bag, Cole Parmer) was fitted on top of the electrode chambers of each reactor to allow release of gases created during electrolysis and microbial reactions. In the EK-Bio reactor, a peristaltic pump (Masterflex L/S) was used to recirculate electrolyte between the anode and cathode compartment at 1mL min^{-1} to manage the pH gradient formed from electrokinetic reactions, an approach commonly used in the field[26].

The soil used for this experiment was a mixture of local Arizona clay topsoil and F85 sand. Soil was added to the central chamber in several layers and compacted with a 0.100 kg hammer with rubber tips. Approximately 6.9 kg of soil were added in total.

The electrolyte was synthetic groundwater at a pH of 8.5 made according to the recipe outlined in previous work modified to include 10 mM sodium bicarbonate, 11.45 mM sulfate, and 2 mM TCE [27]. This synthetic groundwater, free of TCE and sulfate, was periodically added to the EK-Bio reactor through the experiment as electrolyte levels decreased due to electrolysis reactions occurring at the electrodes.

2.2 Reactor Operation After the addition of soil into each reactor, an injection well was created by coring out a 12 mm OD (outer diameter) cylinder with metal tubing and inserting a piece of 6mm OD Teflon tubing with 1mm sized pores. The electron donor, lactate (sodium DL-lactate, 60% syrup, Sigma-Aldrich), was added to each via the injection well to a final groundwater concentration of 10 mM. An incubation stage of 21 days followed this addition to allow anaerobic conditions to be reached. In the case of the EK-Bio reactor, a potential of 30 V was applied to distribute the lactate during this time leading to a current that stabilized around 10 mA . With the electrodes 40 cm apart and just under 20 cm in length, this results in a current density of 0.0125 mA cm^{-2}. This current density is in line with work conducted in the field with a current density of approximately 0.0184 mA cm^{-2} (26). According to Cox et al. (2008), this 150 m^2 field site was treated successfully over 14 months with power requirements equivalent to that of two 100-watt light bulbs. This value is relatively low, especially when compared to other remedial technologies, like thermal treatments (26) .

Once anaerobic conditions were reached, as quantified by measurements of oxidation reduction potential (ORP), a bioaugmentation culture known as ZARA-10 was injected into the reactors. The culture was enriched as outlined in Delgado et al[28]. In the EK-Bio reactor, the current was paused during the addition of the culture to allow the culture to acclimate and was resumed after 14 days. A second dose of lactate was added after this time. Both reactors were operated for a total of 11 weeks. Samples were taken approximately weekly to monitor pH, ORP, and concentrations of chlorinated solvents, sulfate, sulfide, lactate, and fermentation products.

2.3 Chemical Analysis After pore-water samples were extracted with a glass syringe, the oxidation reduction potential (ORP) and pH of the samples were measured with probes (Sartorius pHCore). High performance liquid chromatography (HPLC) and ion chromatography (IC) were used to measure lactate and volatile fatty acid (VFA) concentrations and sulfate concentrations after filtration through a 0.2μm PVDF filter. The instruments used were a Shimadzu HPLC (LC 20-AT) with an Aminex HPX-87H (Bio-Rad) column and photodiode-array detector (210nm) and a Metrohm 930 Ion Chromatograph with a Metrosep A Supp 5-150/4.0 column and A Supp 5 100x carbonate based eluent. Total soluble sulfides were measured with the HACH kit TNT861, and hydrogen sulfide gas was measured with Draeger tubes (MSI-Mid State Instruments LLC). A gas chromatograph (GC) (Shimadzu) equipped with a flame ionization detector (FID) with a packed column (Restek Rtx-624) was used to measure TCE and daughter products. Liquid samples of 1 mL were withdrawn from each port and placed in a 2mL capped vial. After 24 hours of shaking, headspace samples were withdrawn from the vials with a 500 μl gas-tight syringe, and 200μl of gas was injected into the GC for analysis. Scanning electron microscopy energy dispersive X-ray spectroscopy (SEM-EDX) (Nova 200 NanoLab) at the Arizona State University (ASU) Eyring Materials Center was used to detect insoluble mineral compounds present in the soil at the end of the experiment.

2.4 Microbial Community Analysis At the end of the experiment, vertical soil cores were taken along each sampling port. DNA was extracted from the soil samples using the MoBio Powersoil® DNA isolation kit. The Qiagen DNeasy PowerClean Pro Cleanup kit was then used to further improve the quality of the DNA.

The barcoded primer set 515/806R was used to perform sample sequencing on the V4 region of the 16S rRNA gene [29,30]. Library preparation was conducted using a protocol from the Earth Microbiome Project at the Microbiome Analysis Laboratory in the Biodesign Swette Center for Environmental Biotechnology, Arizona State University [31]. A MiSeq Illumina sequencer (Illumina Inc., Dan Diego, CA) was used for the sequencing via the chemistry version 2 (2 x 150 pair-end). Demultiplexed paired-end fastq files produced by CASAVA (Illumina) were used as inputs to QIIME2 version 2020.2 for evaluation [32,33]. Fastq files were quality filtered, trimmed, denoised, and merged with the DADA2 software package wrapped in QIIME2 [34]. Sequences were truncated at 250 basepairs due to a decline in quality of reverse reads that point. The QIIME2 feature-classifier plugin and the Naïve Bayes classifier trained on the Greengenes 13.8 99% OTU full-length sequences were used to assign taxonomy. Alpha and beta-diversity analysis was completed with the QIIME2 q2-diversity plugin at a sampling depth of 8,750. A pairwise PERMANOVA test of beta diversity significance using weighted unifrac distance was run in Qiime2 using the beta-group-significance command, and the Kruskal-Wallis test of alpha diversity significance was run in Qiime2 using the alpha-group-significance command. Raw sequences for this project are available in the NCBI SRA under the BioProject ID PRJNA631539.

3. Results and discussion

3.1 EK-Bio Treatment Outperformed Traditional Bio Method Figure 2 shows the transformation of TCE to daughter products across the Bio and EK-Bio reactors over the 11-week experiment. In the Bio reactor, products of microbial reductive dechlorination, cis-DCE and VC, appeared by week 4. Trace amounts of non-toxic ethene, 2.1-4.6 μM, appeared by week 8, but the largest concentration of daughter products remained cis-DCE and VC with average concentrations across the reactor of 86.5 μM (58.4% deviation) and 17.5 μM (52.0% deviation) respectively. Hindrance of microbial reductive dechlorination leading to cis-DCE accumulation has been reported in cultures where *Dehalococcoides* is out competed by other microbes for H_2 [28].

In the EK-Bio reactor, microbial reductive dechlorination products appeared at week 4, similar to the Bio reactor. There was also an initial spike in TCE at week 2, likely due desorption caused by electroosmosis[35]. Minimal amounts of the reductive dechlorination daughter product cis-DCE were observed in the EK-Bio reactor, but spikes of VC, 17.7 μM, were detected by week 11. Near the end of the experiment at week 9.5, ethene concentrations in the EK-Bio reactor (28.3 μM) were higher than the Bio reactor (4.6 μM). Acetylene, a TCE daughter product formed through reaction with mineral compounds or cathodic reduction, appeared in the cathode chamber of the EK-Bio reactor early on in the experiment at a concentration of 1.2 mM and reached 1.4 mM and 1.9 mM at ports 1 and 2 by week 11. By the end of the experiment acetylene concentration across the reactor averaged 824.0 μM (45.5% deviation). These results suggest both biological and chemical transformation of TCE occurred in the EK-Bio reactor as acetylene is the signature product of abiotic reaction while VC is a signature daughter product of microbial reductive dechlorination found infrequently in abiotic reactions [20].

3.2 Conditions for Microbial Reductive Dechlorination Eventually Achieved in Both Reactors Differing dechlorination results can be attributed to variations in substrate transport rates and operating conditions in each reactor. In the Bio reactor, conditions for microbial reductive dechlorination were reached at a slower pace than in the EK-Bio reactor. A negative ORP reflective of anaerobic conditions was achieved in the cathode side of the Bio reactor near the injection port by week 3, prior to injection of bioaugmentation culture, but reducing conditions were reached in the anode side only by week 9. Acetate, the fermentation product of lactate and an indication of anaerobic conditions, was not measurable in the Bio reactor until week 6. Concentrations of acetate remained low, < 13 mM, until week 10 when increased concentrations were measured in the anode and cathode chambers, 43.9 and

38.5mM respectively. While more time was required to reach reducing conditions and transport substrate, pH remained near neutral in the Bio reactor for the duration of the experiment.

Contrastingly, in the EK-Bio reactor conditions for microbial reductive dechlorination were reached quickly, though with greater challenges for pH control. A negative ORP was achieved by week 2, immediately following injection of lactate in week 1. Detection of lactate was delayed until week 8, but once measurable, was present at high concentrations, approximately 1-2mM, and evenly distributed throughout the contaminated soil. Concentrations of acetate peaked at week 10, around 60 mM. This increase in acetate, seen in both reactors at week 10, may have been due to uneven flow paths leading to areas of high concentration or to acetate produced via inorganic carbon and hydrogen via acetogenesis (Figure 4). Particularly in the EK-Bio reactor, the high relative abundance of these genera at port 4 corresponds to acetate peaks at week 10 near the anode chamber port 4. A large pH gradient developed in the EK-Bio reactor by week 2 (Figure 5) but was neutralized by slightly increasing the rate of recycle between the anode and cathode. This gradient reappeared by week 10 suggesting an even greater recycling rate or added buffer might be needed. While pH management was more difficult in the EK-Bio reactor, reducing conditions were achieved earlier due to better distribution of lactate, creating conditions that were more amenable to microbial reactions than in the Bio reactor.

3.3 Sulfate Transport and Abiotic Reactions Effect Treatment Performance Despite eventually reaching reducing conditions and diffusion of electron donor throughout the reactor, microbial reductive dechlorination stalled in the Bio reactor at cis-DCE and VC between weeks 9 and 10. This stall can be attributed to competition between Dehalococcoides and SRB due to sulfate transport limitations. As seen in Figure 6, in the Bio reactor, sulfate remained distributed throughout the soil for the duration of the experiment. By the end of the experiment, concentrations of sulfate remained over 3 mM at some locations possibly leading to competition for H2 by SRB. The competition facing *Dehalococcoides* was further confounded by with increasingly inhibitory concentrations of sulfide, already at soluble concentrations of up to 0.2mM[17]. Contrastingly, by week 3 in the EK-Bio reactor sulfate nearly disappeared except in the anode and cathode chambers. With an expected rate of electromigration of 1.2 x 10-6 m s^{-1}, sulfate would be transported the length of the reactor in 4.5 days (calculations in SI). The accumulation of sulfate in the anode and cathode chambers by week 3 is reflective of this quick migration rate. Competition for H_2 between SRB and Dehalococcoides was quickly eliminated, allowing microbial reductive dechlorination to ethene to proceed uninhibited.

The reduction of sulfate in the EK-Bio reactor may have also contributed to formation of TCE daughter product acetylene. Hydrogen sulfide generated from microbial sulfate reduction (equation 3) can react with iron oxide/hydroxide species to form elemental sulfur and iron (II) sulfide (equation 4). The iron (II) sulfide subsequently reacts with TCE to form acetylene (equation 5), as outlined in the reactions below[36]. Sulfate removal of up to 75%, along with low soluble sulfide concentrations measured (Figure 6), suggest precipitation of metal sulfides. Transformations of chlorinated ethenes via this biogeochemical pathway have been reported in lab studies and in the field through monitored natural attenuation schemes or engineered systems[36–38]. Measurements of insoluble iron species by the SEM-EDX averaged 3.8% by weight, indicating a high enough concentration to transform available TCE to acetylene.

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad (3)$$

$$2FeOOH + 2H_2S \rightarrow 2FeS + S^\circ + 4H_2O \quad (4)$$

$$4FeS + 9C_2HCl_3 + 28H_2O$$

$$\rightarrow 4Fe(OH)_3 + 4SO_4^{2-} + 9C_2H_2 + 27Cl^- + 35H^+ \quad (5)$$

Generally, the rate of microbial reductive dechlorination has been reported to be much faster than abiotic reaction with iron minerals[20]. However, the rate of abiotic reaction can be significantly increased at sites with favorable environmental conditions which increase reactant loading, as occurred in the EK-Bio reactor[20]. In addition to high concentrations of organic carbon, iron, or sulfate, the abiotic reaction rate can be accelerated with increases in pH[39]. Weerasooriya and Dharmasena [39] demonstrated a monotonic increase in reaction rate between iron (II) sulfide and TCE from 0.03 h^{-1} at pH 8 to over 0.05 h^{-1} at pH 10. While the pH spikes in the EK-Bio reactor were detrimental to microbial reductive dechlorination, they may have aided reaction rates of biogeochemical transformation. While both the EK-Bio and Bio reactors had high levels of sulfate and iron needed to generate the reactive chemical species, but quicker attainment of reducing conditions and more uniform organic carbon substrate distribution in the EK-Bio reactor may have better facilitated biogeochemical reduction of TCE.

Alternatively, acetylene can be generated through direct cathodic reduction of TCE. Cathodic reduction of chlorinated solvents has been investigated previously with Pt, Pd, iron, and graphite electrodes in closed recirculation systems [23–25]. TCE can follow several abiotic dechlorination pathways with multiple daughter products, but the appearance of acetylene indicates β-elimination was likely the mechanism. The use of a graphite electrode has been reported to lead to the by-product chloromethane, a known carcinogen, through the combination of chloride and methyl radicals created through the Kolbe reaction of acetate [25]. No chloromethane was detected in this experiment, likely as acetate was consumed by SRB or dechlorinating bacteria.

Under similar conditions in a soil free reactor with a granular graphite electrode and the application of 15 V, Al-Abed and Fang (2007) measured transformation of 76% of TCE to ethene and ethane in 25 hours. In this EK-Bio experiment, acetylene may have been the primary reaction product rather than ethene or ethane as it volatilized out of solution into the gas bag, preventing further reaction with the cathode or iron species in the soil. The early appearance of acetylene in the cathode chamber of the EK-Bio reactor and the complete absence of acetylene in the Bio reactor suggest cathodic reduction was the primary, or at least initial, abiotic transformation mechanism of TCE in this experiment[25]. The rate of electroosmosis, the primary transport mechanism for acetylene, is 2.9 x 10^{-7} m s^{-1} (calculations in SI). At this rate, which is similar to those previously reported, acetylene generated in the cathode chamber by cathodic reduction could travel to the anode chamber in approximately 16 days[40]. The

appearance of acetylene in the anode chamber at week 6 could be due to cathodic reduction in earlier weeks and subsequent electroosmostic transport throughout the experiment. Thus, the presence of acetylene throughout the reactor does not necessarily imply a production through FeS minerals. In fact, the early appearance of acetylene within the cathode chamber suggests that cathodic reduction was likely the primary, if not sole, abiotic transformation mechanism.

3.4 Microbial Community Analysis Reveals Large Shift in EK-Bio Reactor Analysis of the final resulting microbial community structures shows differences between the native microbial community, the Bio reactor samples, and the EK-Bio reactor samples. The initial soil samples are very similar to those in the Bio reactor. The samples from the EK-Bio reactor are very different from the initial soil samples (P = 0.020) or the samples from the Bio reactor (P = 0.001), especially near port 4. These data and the matrix distances can be seen in the weighted unifrac beta (between sample) diversity plot in in Figure 7A. In weighted unifrac analysis, the most abundant taxa drive differences between the distance matrices. The differences between samples from the EK-Bio reactor and others is likely due to the larger increase in fermentative and sulfate reducing bacteria, this is illustrated in more detail by our taxonomic analysis in Figure 8. Further, the microbial community appeared to be most different at port 4 of the EK-Bio reactor, which is consistent with the taxonomy data showing an increase in phylotype similar to Bacilli at this location (Figure 8).

Alpha (within sample) diversity in the Bio reactor was largely unchanged from that of the initial soil microbial community (P = 0.79; Kruskal-Wallis test), but significantly decreased in the EK-Bio reactor (P = 0.038; Kruskal-Wallis test), especially near ports 1 and 4 (Figure 7B). Average operational taxonomic units (OTUs) in the initial soil sample were 803 versus 842 in the Bio reactor and 449 in the EK-Bio reactor. This type of decrease in alpha diversity has been previously reported in the literature and attributed to secondary effects of EK like changes in pH, which is consistent with chemical data reported here in week 10 (Figure 5)[41–43]. This drop can also be linked to the enrichment and increased abundance of one microbe, in this case, phylotypes most similar to the class Clostridia.

Assessment of the microbial community members shows many similarities between the Bio and EK-Bio reactors[28]. Both reactors displayed comparable levels of the phylotype most similar to dechlorinating bacteria, though phylotypes most similar those found in the overall enrichment culture were slightly more elevated in the EK-Bio reactor. The microbial community of the enrichment culture was previously described in Delgado et al. 2014 and includes, among others, Clostridia, Bacteroidia, Anaerolineae, and Dehalococcoidetes [28]. Clostridia, Bacteroidia, and Anaerolineae are known to contain fermentative bacteria and are frequently found in the environment[44–46]. The class Clostridia is also known to contain species of SRB[47]. Phylotypes similar to the classes Clostridia, Bacteroidia, and Anaerolineae, constituted on average, 27% of the community in the Bio reactor and 43% of the community in the EK-Bio reactor (Figure 8). Phylotypes similar to the class Dehalococcoidetes, which contains the TCE dechlorinating *Dehalococcoides*, made up 0.1% or less of each community in both reactors, but were slightly more abundant in the Bio reactor. Both reactors also displayed similar levels of the phylotype most similar to the class Deltaproteobacteria, which is known to also contain SRB and iron reducing bacteria[47]. Unlike the Bio reactor, there was also a significant enrichment of phylotypes most similar to the class Bacilli near port 4 of the EK-Bio reactor. Analysis on a genus level (not shown) indicates this was most similar to the phylotype *Ammoniphilus*, an aerobic haloalkalitolerant organism[48]. Enrichment of this organism near port 4 may have been due oxygen production near the anode. The relative abundance of phylotypes most similar to genera known to have homoacetogenic metabolisms can be seen in Figure 4. Overall, these microbial community results support the chemical data which indicate microbial reductive dechlorination facilitated by a bioaugmentation culture occurred in both reactors.

4. Conclusion

Electrokinetic and traditional bioremediation approaches both resulted in transformation of TCE in a clay soil matrix with high sulfate concentrations. Microbial reductive dechlorination was the primary mechanism in the Bio reactor with a traditional bioremediation approached. These microbial reactions stalled at the end of the experiment, likely due to competition for H_2 caused by SRB or inhibitory effects of the sulfate reduction product sulfide [17]. Greater transformation of TCE occurred in the EK-Bio reactor, where acetylene was the primary daughter product, indicating the dominance of an abiotic mechanism, either biogeochemical reaction or direct cathodic reduction. The production of acetylene near the cathode during the first few weeks of experiment in the EK-Bio reactor strongly suggest that electrochemical reduction was the major mechanism of TCE reduction. The appearance of VC and ethene in the EK-Bio reactor indicates microbial reductive dechlorination occurred as well, though as a secondary transformation mechanism. Taxonomic analysis showed enrichment of phylotypes similar to those reported in the dechlorinating inoculum in each reactor, supporting the conclusion that microbial reduction dechlorination occurred in both reactors, though to different extents [28]. Results of microbial community structure analysis are very similar to previously published work which reports some decreases in alpha diversity and beta diversity along the treatment zone [41,49,50]. The results of this experiment show that the combined biotic and abiotic mechanisms of EK-Bio can result in improved remediation over traditional bioaugmentation methods. These two mechanisms can act synergistically with microbial reductive dechlorination consuming acetate to prevent electrochemical generation of the carcinogen chloromethane and with abiotic formation of acetylene from TCE acting as a fermentable substrate for microbial reactions. Results of this work demonstrate EK-BIO can be considered a feasible remedy for chlorinated solvent contaminated environments with transport limitations and geochemical challenges, thus extending much needed treatment to a great number of impacted water sources.

Conflicts of interest

There are no conflicts to declare

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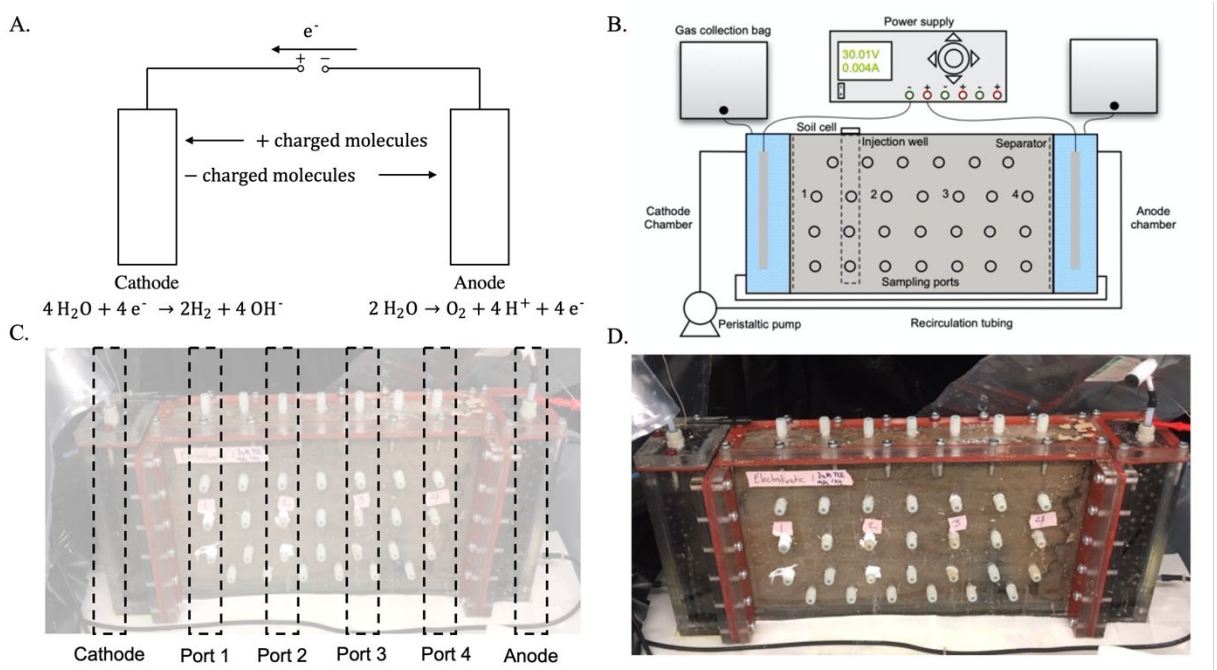

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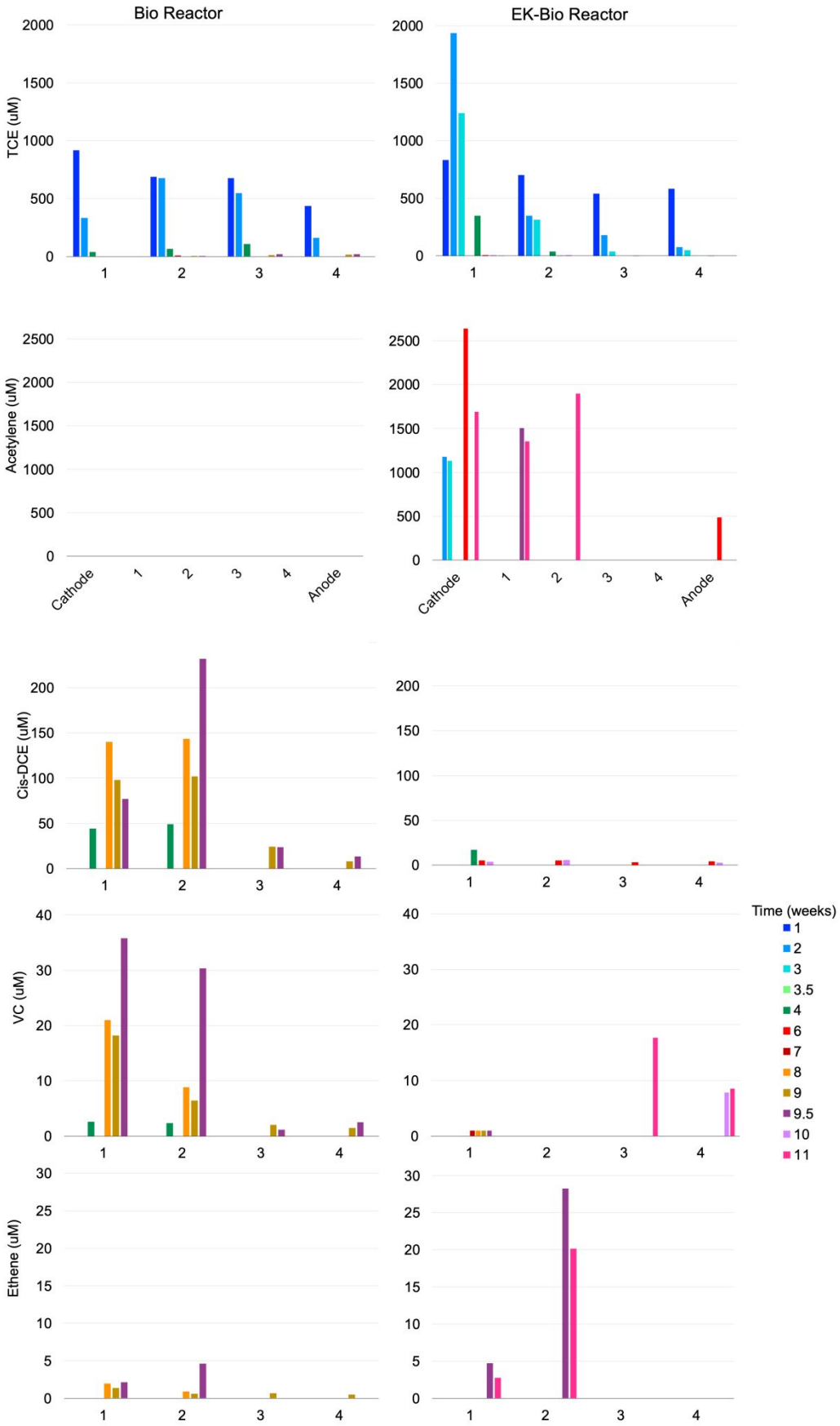

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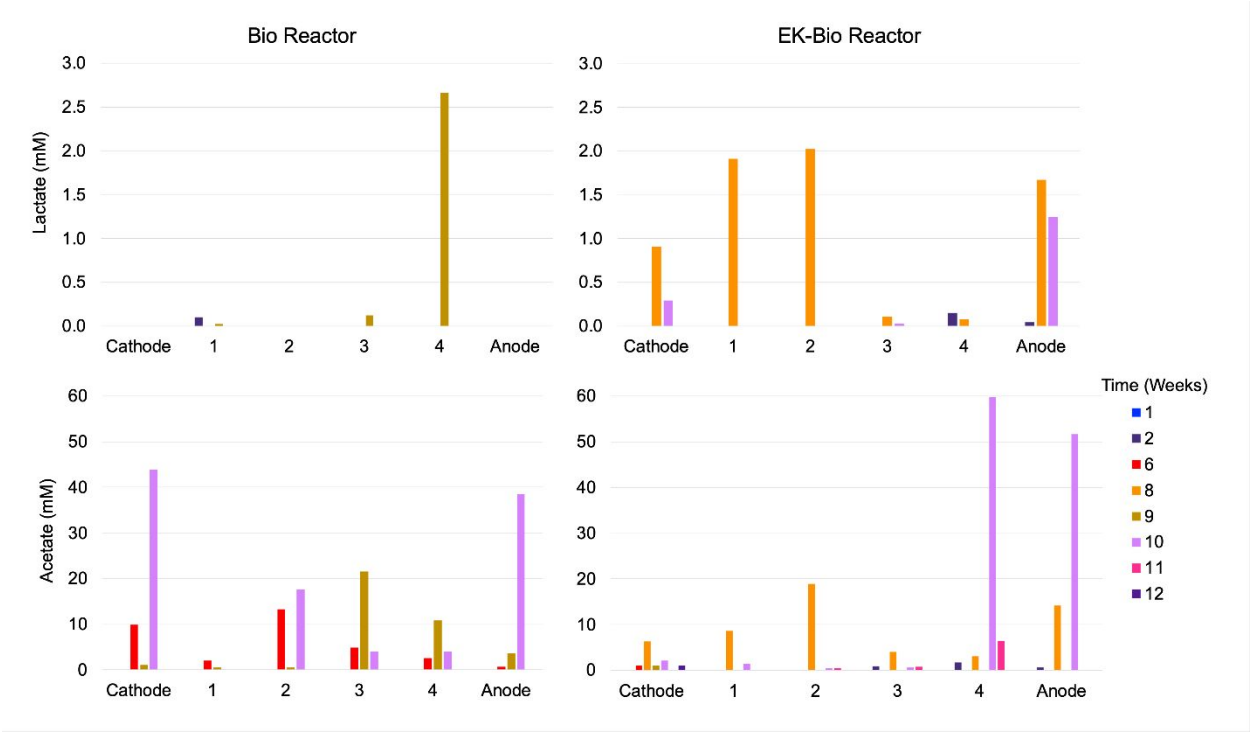

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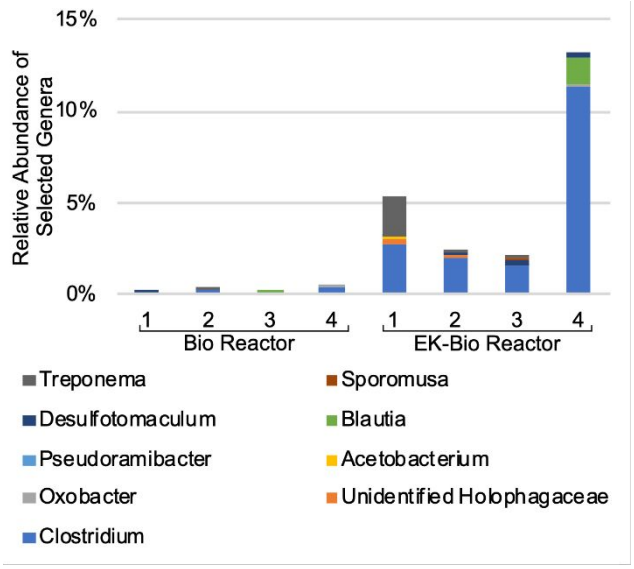

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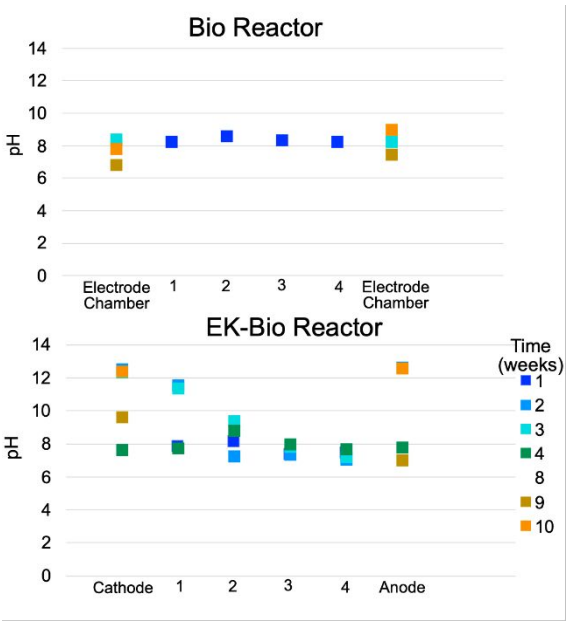

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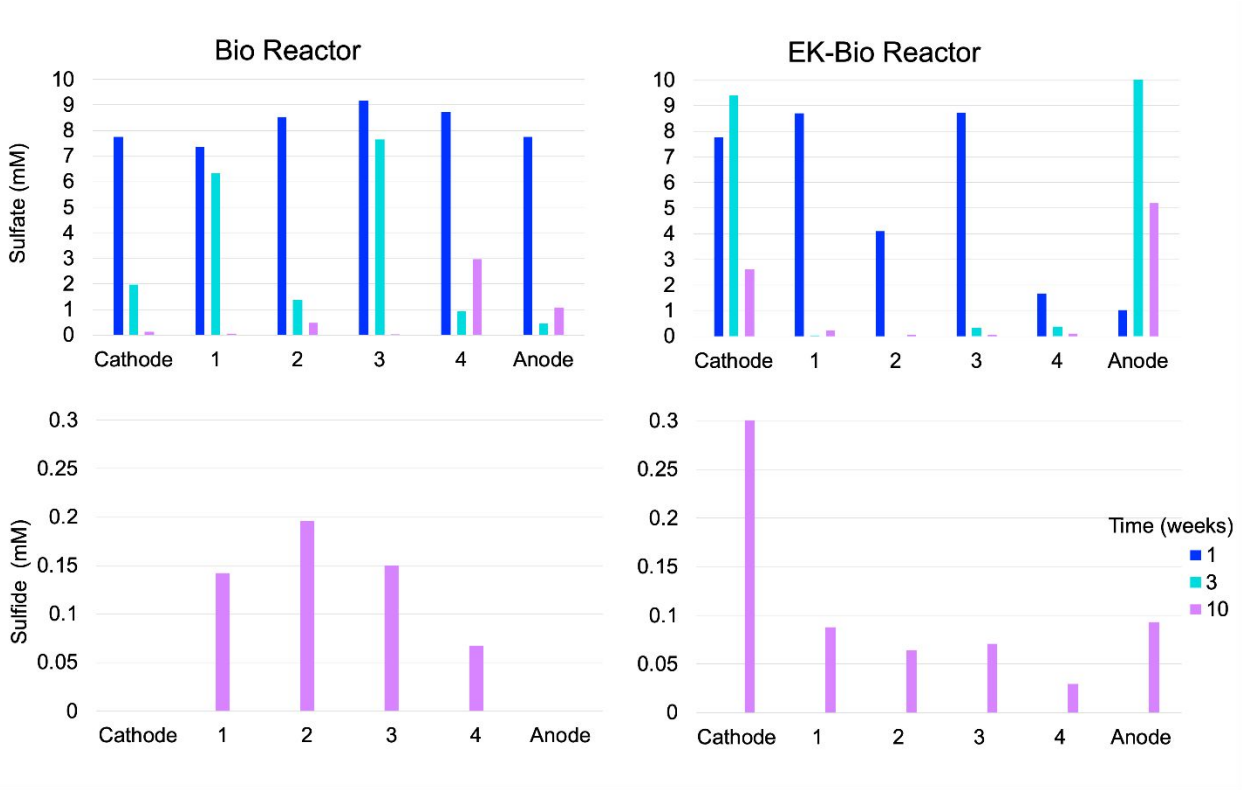

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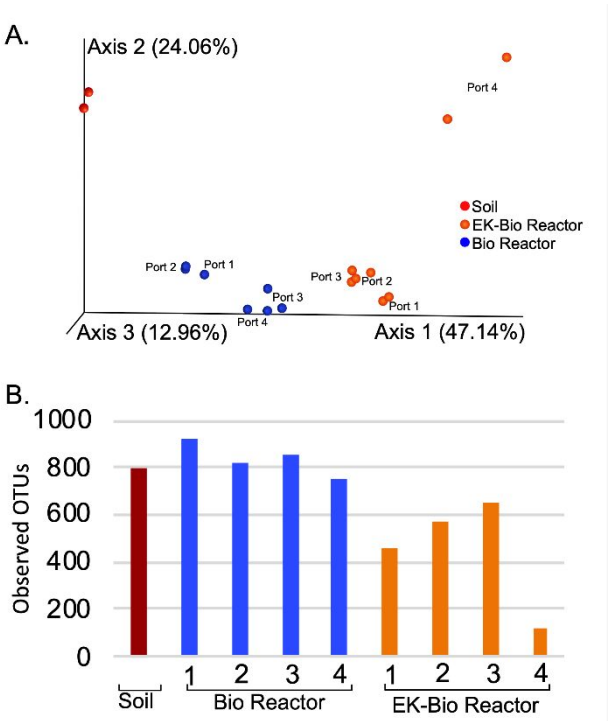

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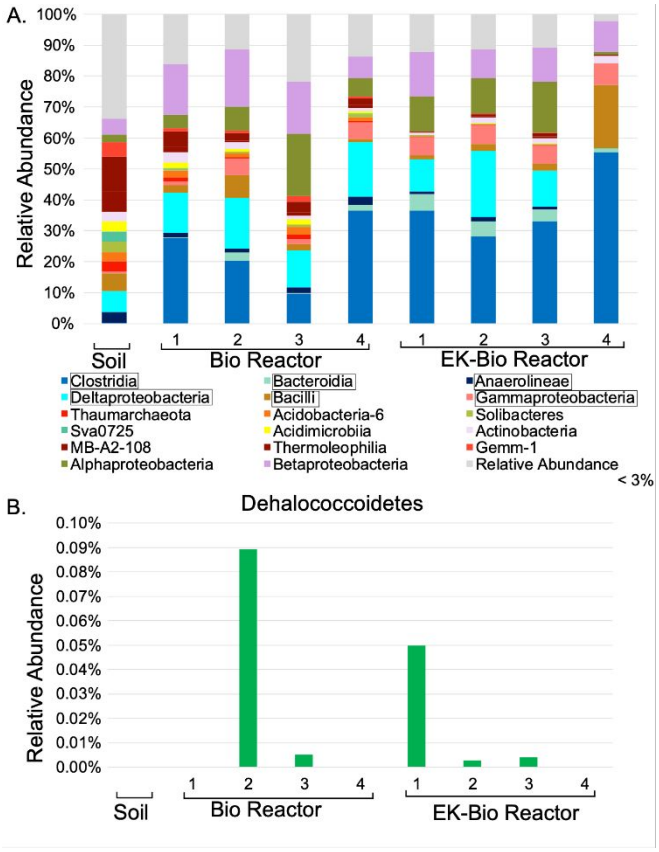

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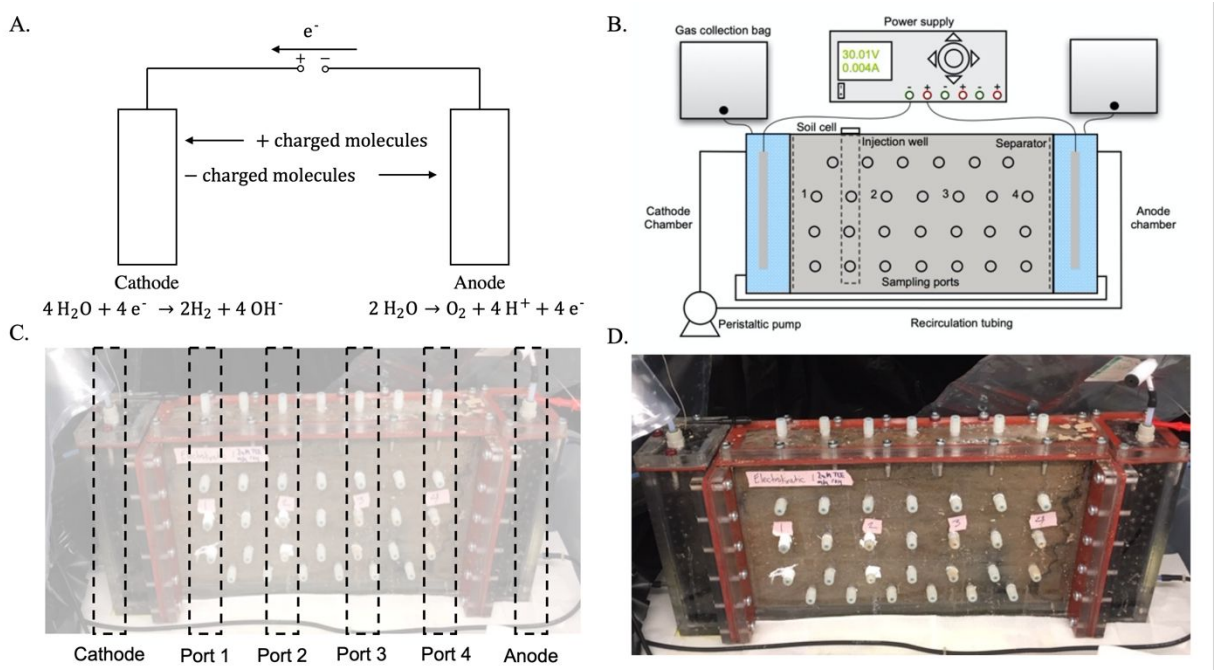

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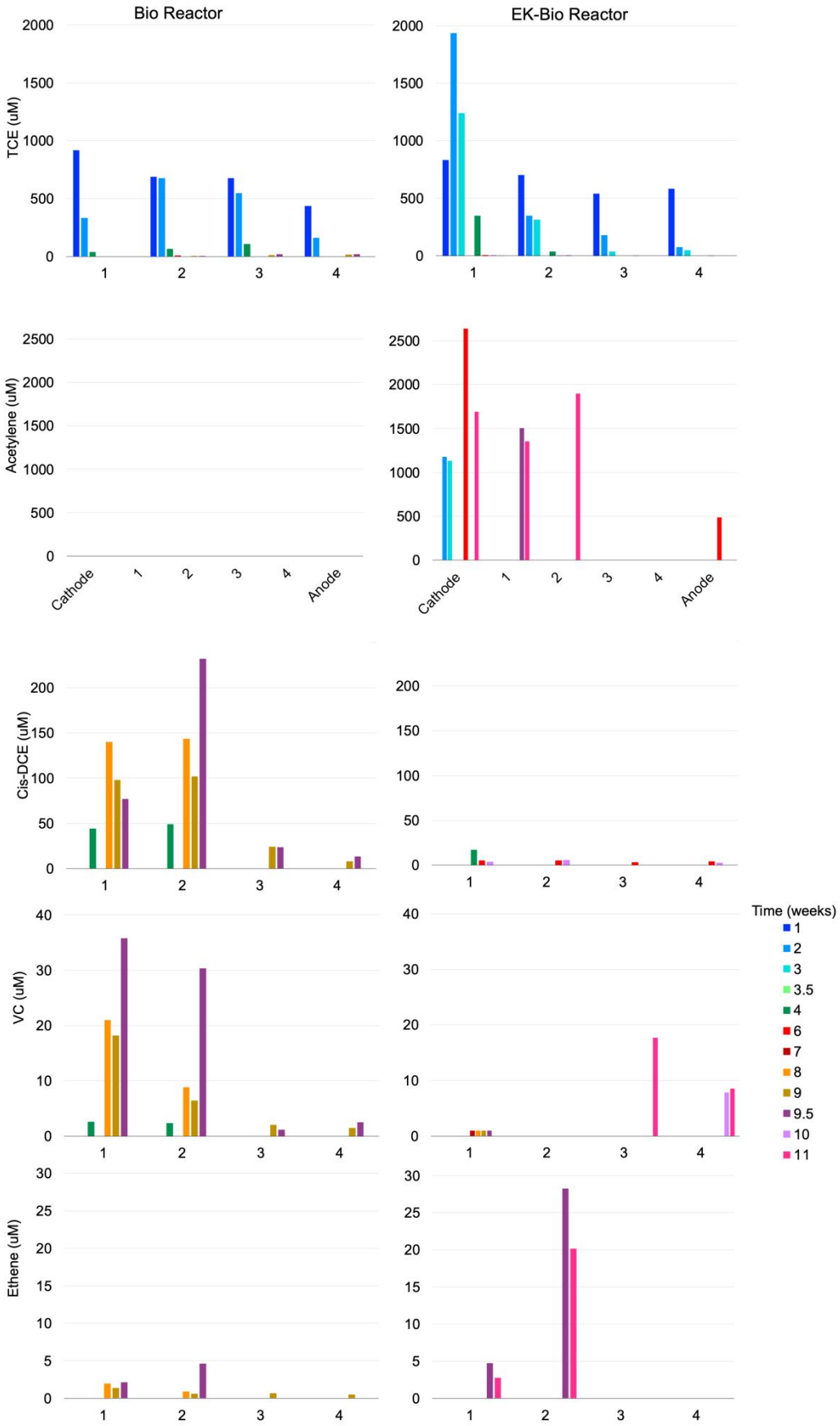

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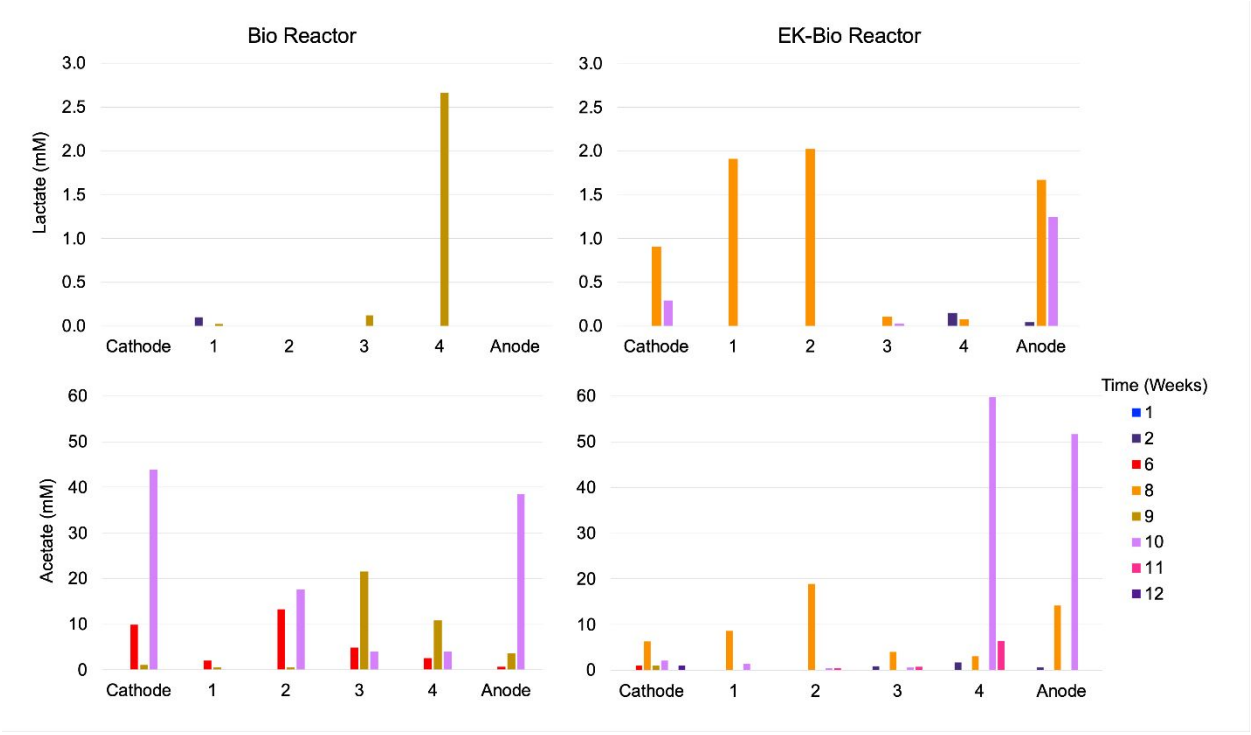

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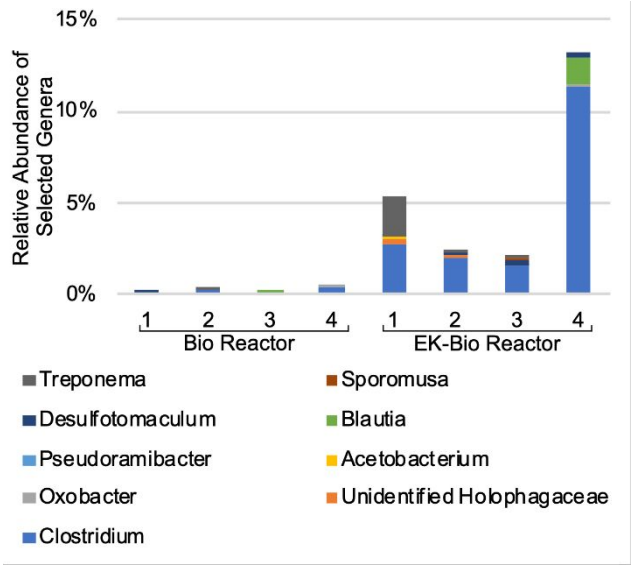

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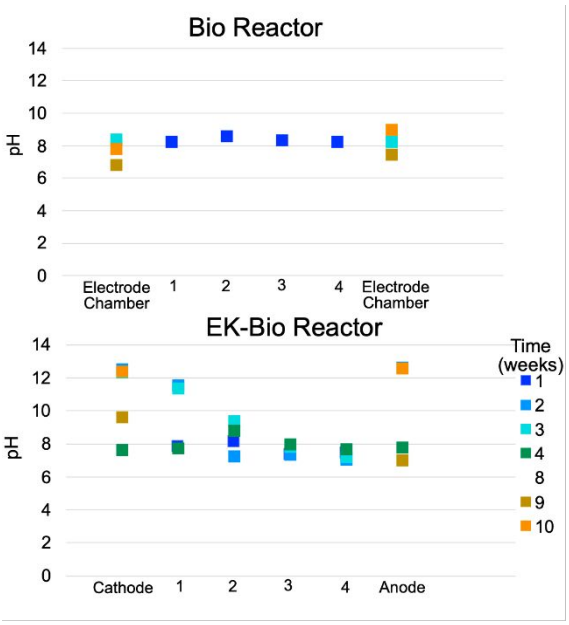

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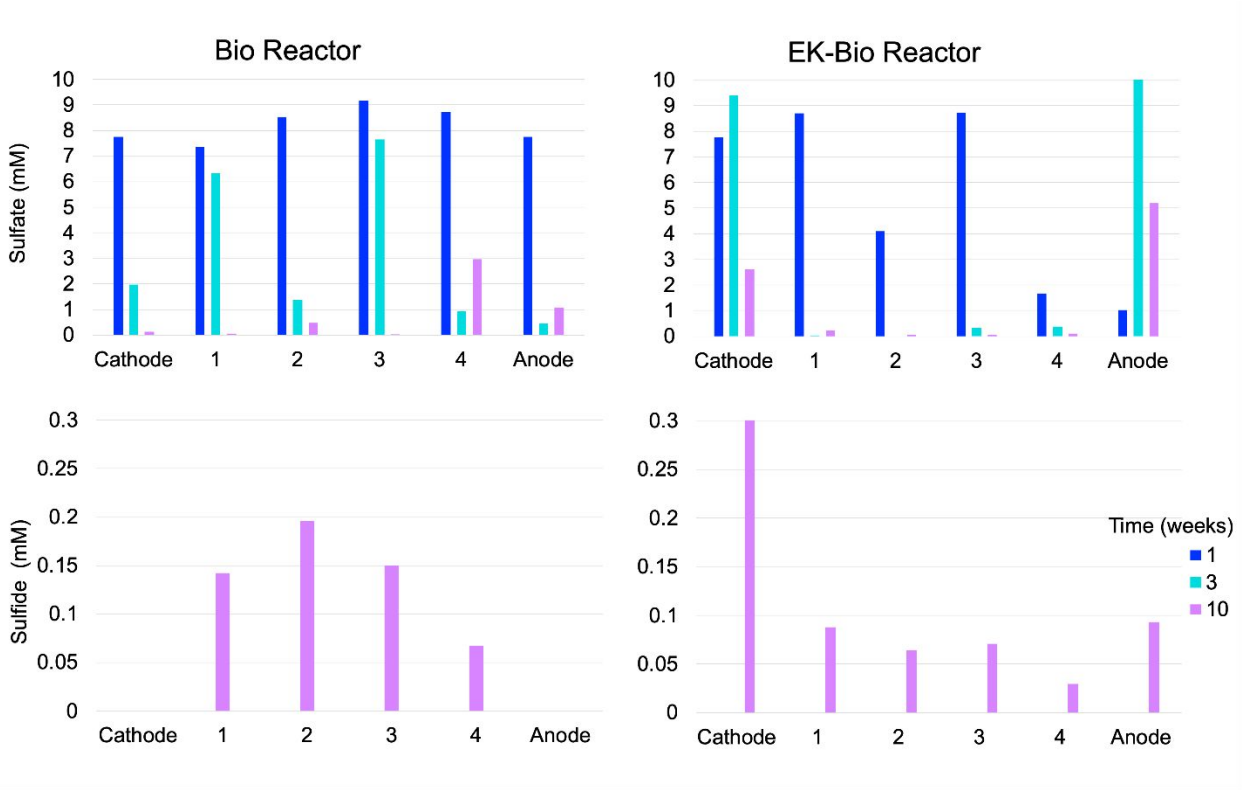

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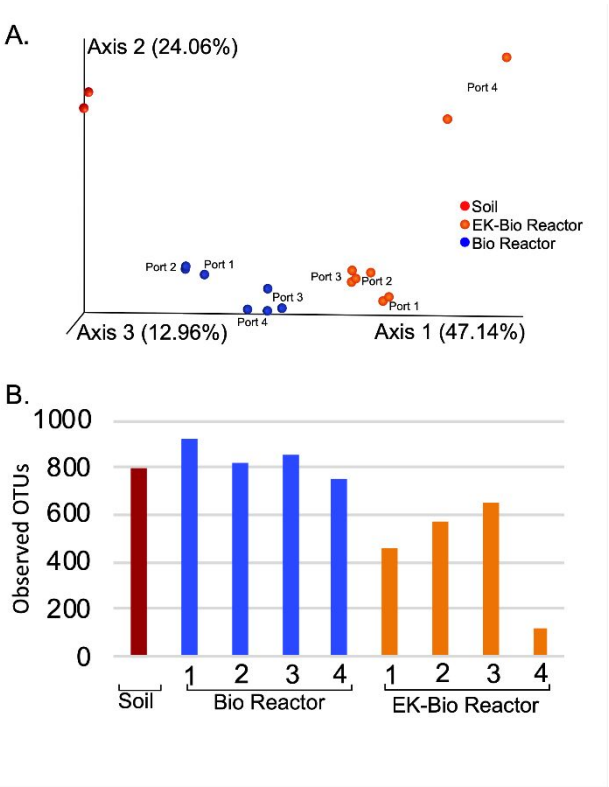

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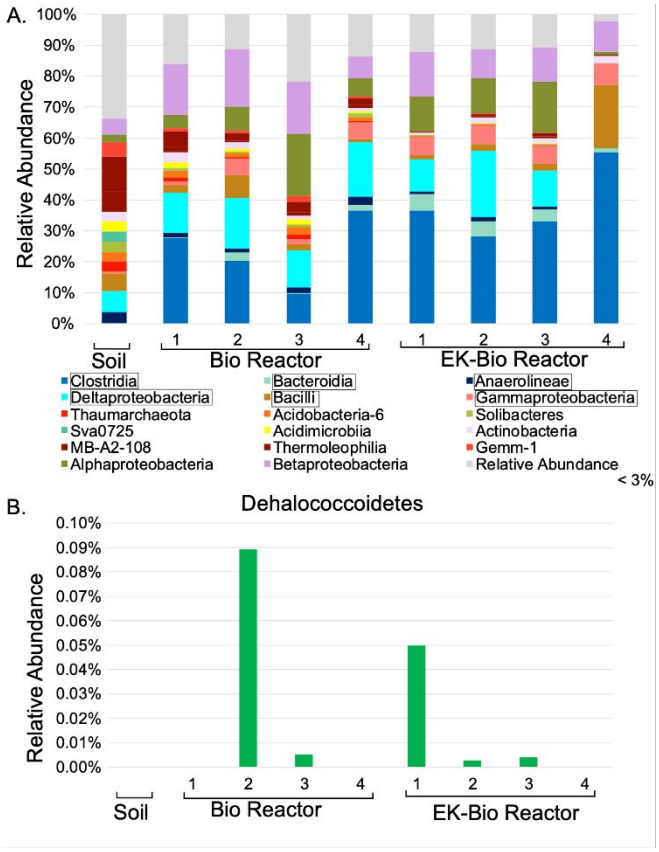

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Journal Name ARTICLE

Coupled electrokinetic and biological remediation method leads to improved treatment of chlorinated solvents at high sulfate, transport limited sites

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Chlorinated solvents are some of the most pervasive pollutants found in groundwater and drinking water sources in the United States (U.S.). In the early 2000s, bioremediation emerged as a novel and effective technology, but was limited by challenges to delivery and transport of nutrients and microbes. Electrokinetic bioremediation (EK-Bio) has since emerged as a promising alternative to solve these limitations, delivering successful results at the lab and pilot scale. EK-Bio can be applied at sites where traditional bioaugmentation, the transformation of pollutants via an added microbial culture, is transport limited. The application of direct current *in situ* in electrokinetic (EK) remediation facilitates transport of the microbial culture and substrate in the subsurface. Despite this recent surge in interest surrounding EK-Bio, it is not clear how this technology would perform at a site with elevated levels of alternative electron acceptors, another common barrier to successful bioremediation. Our objectives were to use bench scale reactors to 1) determine which reactions and processes would dominate when using EK-BIO to treat TCE contamination at a site with high levels of the alternative electron acceptor sulfate, 2) compare EK-Bio to a traditional bioremediation application without electrokinetics, and 3) understand the effect of EK-Bio on the microbial community under these conditions. Our results showed complete transformation of TCE to ethene and acetylene by EK-Bio, while only 15% of TCE was transformed to cis-DCE and VC via traditional bioaugmentation. Instead, the majority of the TCE was converted to acetylene, likely due to its electrochemical reduction at the cathode. EK-Bio out performed traditional methods as it facilitated TCE biotic and abiotic transformation. Next generation sequencing analysis showed the microbial community in the EK-Bio reactor was highly enriched by the bioaugmentation culture, and community structure and diversity were minimally affected by the electrokinetic application. These results demonstrate that EK-Bio is an effective and promising remedy for treating chlorinated solvent contamination at transport limited sites with high concentrations of competing electron acceptors. This combined treatment strategy can be used to extend traditional bioaugmentation to a greater number of polluted sites, restoring more contaminated water systems for beneficial use.

Water Impact Statement

Trichloroethene (TCE) is one of the most widespread contaminants in groundwater affecting an estimated 4.5-18% of drinking water sources in the United States. Combined remediation technologies are required to address the increasingly complex sites which remain polluted. Here, we present a combined bioelectrochemical approach which improves treatment outcomes and extends applications of traditional technologies.

1. Introduction

Chlorinated solvents like perchloroethylene (PCE) and trichloroethylene (TCE) are common groundwater contaminants throughout the United States (U.S.) which cause concern due to their toxic properties and widespread occurrence [1,2]. Previously used as dry cleaning and degreasing agents, these chemicals entered the watershed due to accidental spills and improper disposal [3,4]. PCE has been detected in 4% of aquifers tested by the U.S. Geological Survey (USGS), and TCE has been measured in 4.5-18% of the country's drinking water supply sources[5,6]. Health issues associated with PCE and TCE range from damage to the nervous system, liver, kidney, and reproductive systems, to developmental issues, and possibly cancer[5,6]. PCE and TCE daughter product vinyl chloride (VC) is a known carcinogen[7]. Given the health effects associated with these compounds and their daughter products, complete removal or transformation to non-toxic ethene is required to protect human health[8].

One commonly used method for treating chlorinated solvent contamination is bioaugmentation, the *in situ* addition of a bacterial culture capable of dechlorinating PCE and TCE to ethene[9,10]. The key bacteria, *Dehalococcoides*, removes one chlorine atom at time and replaces them with hydrogen in a process known as microbial reductive dechlorination, transforming PCE to TCE, TCE to cis-dichlorethene (cis-DCE), cis-DCE to VC, and VC to ethene[11]. *Dehalococcoides*, are strict anaerobes that use H_2 as an electron donor and acetate as a carbon source [9]. They require moderate temperatures (25-40°C) and neutral pH conditions[11]. In the subsurface, H_2 and acetate can be delivered to *Dehalococcoides* through anaerobic fermentation of substrates like lactate[12]. Bioaugmentation using cultures with *Dehalococcoides* was developed as a treatment strategy in the 1990's, and hundreds of sites have since been successfully treated with this remedy[13]. Despite this success, there remain challenges to bioaugmentation efficacy. Two of the most substantial challenges to anaerobic bioremediation of chlorinated solvents are microbial competition from native soil bacteria and transport of bioaugmentation cultures and substrates *in situ* [14].

One of these challenges, transport limitations, can be addressed by pairing traditional bioaugmentation with technologies that improve delivery of nutrients and microbes, like electrokinetics (EK) [15]. EK is the application of direct current to the subsurface to induce transport *in situ*. Soluble molecules may be transported via movement of fluid through pore spaces (electroosmosis) and ions or other charged molecules may move to the oppositely charged electrode (electromigration and electrophoresis) [15]. When EK is

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combined with bioaugmentation (EK-Bio), the bioaugmentation culture and electron donor are added to the subsurface via traditional injection wells and transported via electrokinetic mechanisms in addition to the natural advective gradient[15]. It is important to note that the application of current causes electrochemical reactions at each electrode, namely production of oxygen gas at the anode and hydrogen gas, H_2, at the cathode according to the reactions below [16].

$$Anode - \ H_2O \rightarrow 2H^+ + \frac{1}{2}O_2 + 2e^- \ \#(1)$$

$$Cathode - \ 2H_2O + 2e^- \rightarrow 2OH^- + H_2 \ \#(2)$$

Further, these reactions generate a pH gradient with acidic conditions at the anode and basic conditions at the cathode[16]. Both the extreme pH fronts and the oxygen produced by the anode must be carefully managed at sites where microbial reductive dechlorination is employed to maintain the specific conditions required by *Dehalococcoides*.

Microbial competition for H_2 in the subsurface between *Dehalococcoides* and native soil bacteria is caused by high concentrations of alternative electron acceptors, like sulfate. High levels of sulfate are often found in PCE and TCE plumes due to natural sources, like atmospheric deposition, sulfate mineral dissolution, and sulfide mineral oxidation, or anthropogenic sources, like coal mines, power plants, and refineries [17,18]. Like microbial reductive dechlorination, microbial sulfate reduction is carried out with H_2 as an electron donor, leading to competition between sulfate reducing bacteria (SRB) and dechlorinating bacteria for the limited H_2 available *in situ* [17]. Further, sulfide inhibition of dechlorination also occurs at high concentrations (greater than 5mM) due to the toxicity of the sulfide species (H_2S and HS^-) produced from sulfate reduction [17]. These inhibitory effects are further exacerbated in the field when sites are flooded with electron donor as toxic sulfide species accumulate [17]. It is not clear how EK-Bio would perform at a site with elevated levels of alternative electron acceptors. Electrokinetic bioaugmentation of chlorinated solvents at sites with high concentrations of sulfate has not been extensively studied. It is possible that EK transport of substrate could favor SRB who can out-compete *Dehalococcoides* for H_2[19]. This scenario could cause a stall of microbial reductive dechlorination or generation of a reactive metal sulfide species capable of abiotic dechlorination[20–22]. A mixture of biotic and abiotic reactions could occur, including some electrochemical transformations of TCE which have been reported in closed recirculation systems with Pt, Pd, iron, and graphite electrodes[23–25]. Our objectives were to 1) determine which reactions and processes would dominate at a TCE contaminated site with high levels of the alternative electron acceptor sulfate, 2) compare EK-Bio to a traditional bioremediation application without electrokinetics, and 3) understand the effect of EK-Bio on the microbial community under these conditions.

2. Materials and methods

2. 1 Reactor Design and Set-up This experiment featured a combined electrokinetic bioaugmentation (EK-Bio) reactor and a traditional bioaugmentation reactor (Bio). The Bio reactor was operated with traditional bioaugmentation methods where diffusion is the main processes for mass transfer. No current was applied to the Bio reactor. The EK-Bio reactor was operated with a combined electrokinetic and bioaugmentation approach. Direct current was delivered to the EK-Bio reactor via power supply (Rigol DP832). Each of the two reactors was constructed from acrylic, consisting of a central soil compartment (40 cm long, 8 cm wide, 20 cm high; 6.4 L) between two electrode chambers (10 cm long, 8 cm wide, 20 cm high; 1.6 L). The soil and electrode compartments were divided with a plastic porous separator (Midland Scientific Inc, HDPE, 1.6mm, medium grade porosity). A grid of nylon Swagelok sampling ports fitted with rubber septa covered the top and front face of each soil chamber. Porous metal tubes made of rolled screening (nickel 200, wire mesh 70 x 70) were inserted into all the ports to allow collection of porewater for sampling. Graphite electrodes (Fine extruded rod, 1.27 cm OD, Graphite Store) were used for both the anode and cathode. Once the electrodes were in place, the electrode chambers were filled with glass beads (11mm OD) to decrease the electrode compartment volumes to approximately 280 mL. A gas bag (5L, PVF Tedlar bag, Cole Parmer) was fitted on top of the electrode chambers of each reactor to allow release of gases created during electrolysis and microbial reactions. In the EK-Bio reactor, a peristaltic pump (Masterflex L/S) was used to recirculate electrolyte between the anode and cathode compartment at 1mL min^{-1} to manage the pH gradient formed from electrokinetic reactions, an approach commonly used in the field[26].

The soil used for this experiment was a mixture of local Arizona clay topsoil and F85 sand. Soil was added to the central chamber in several layers and compacted with a 0.100 kg hammer with rubber tips. Approximately 6.9 kg of soil were added in total.

The electrolyte was synthetic groundwater at a pH of 8.5 made according to the recipe outlined in previous work modified to include 10 mM sodium bicarbonate, 11.45 mM sulfate, and 2 mM TCE [27]. This synthetic groundwater, free of TCE and sulfate, was periodically added to the EK-Bio reactor through the experiment as electrolyte levels decreased due to electrolysis reactions occurring at the electrodes.

2.2 Reactor Operation After the addition of soil into each reactor, an injection well was created by coring out a 12 mm OD (outer diameter) cylinder with metal tubing and inserting a piece of 6mm OD Teflon tubing with 1mm sized pores. The electron donor, lactate (sodium DL-lactate, 60% syrup, Sigma-Aldrich), was added to each via the injection well to a final groundwater concentration of 10 mM. An incubation stage of 21 days followed this addition to allow anaerobic conditions to be reached. In the case of the EK-Bio reactor, a potential of 30 V was applied to distribute the lactate during this time leading to a current that stabilized around 10 mA . With the electrodes 40 cm apart and just under 20 cm in length, this results in a current density of 0.0125 mA cm^{-2}. This current density is in line with work conducted in the field with a current density of approximately 0.0184 mA cm^{-2} (26). According to Cox et al. (2008), this 150 m^2 field site was treated successfully over 14 months with power requirements equivalent to that of two 100-watt light bulbs. This value is relatively low, especially when compared to other remedial technologies, like thermal treatments (26) .

Once anaerobic conditions were reached, as quantified by measurements of oxidation reduction potential (ORP), a bioaugmentation culture known as ZARA-10 was injected into the reactors. The culture was enriched as outlined in Delgado et al[28]. In the EK-Bio reactor, the current was paused during the addition of the culture to allow the culture to acclimate and was resumed after 14 days. A second dose of lactate was added after this time. Both reactors were operated for a total of 11 weeks. Samples were taken approximately weekly to monitor pH, ORP, and concentrations of chlorinated solvents, sulfate, sulfide, lactate, and fermentation products.

2.3 Chemical Analysis After pore-water samples were extracted with a glass syringe, the oxidation reduction potential (ORP) and pH of the samples were measured with probes (Sartorius pHCore). High performance liquid chromatography (HPLC) and ion chromatography (IC) were used to measure lactate and volatile fatty acid (VFA) concentrations and sulfate concentrations after filtration through a 0.2μm PVDF filter. The instruments used were a Shimadzu HPLC (LC 20-AT) with an Aminex HPX-87H (Bio-Rad) column and photodiode-array detector (210nm) and a Metrohm 930 Ion Chromatograph with a Metrosep A Supp 5-150/4.0 column and A Supp 5 100x carbonate based eluent. Total soluble sulfides were measured with the HACH kit TNT861, and hydrogen sulfide gas was measured with Draeger tubes (MSI-Mid State Instruments LLC). A gas chromatograph (GC) (Shimadzu) equipped with a flame ionization detector (FID) with a packed column (Restek Rtx-624) was used to measure TCE and daughter products. Liquid samples of 1 mL were withdrawn from each port and placed in a 2mL capped vial. After 24 hours of shaking, headspace samples were withdrawn from the vials with a 500 μl gas-tight syringe, and 200μl of gas was injected into the GC for analysis. Scanning electron microscopy energy dispersive X-ray spectroscopy (SEM-EDX) (Nova 200 NanoLab) at the Arizona State University (ASU) Eyring Materials Center was used to detect insoluble mineral compounds present in the soil at the end of the experiment.

2.4 Microbial Community Analysis At the end of the experiment, vertical soil cores were taken along each sampling port. DNA was extracted from the soil samples using the MoBio Powersoil® DNA isolation kit. The Qiagen DNeasy PowerClean Pro Cleanup kit was then used to further improve the quality of the DNA.

The barcoded primer set 515/806R was used to perform sample sequencing on the V4 region of the 16S rRNA gene [29,30]. Library preparation was conducted using a protocol from the Earth Microbiome Project at the Microbiome Analysis Laboratory in the Biodesign Swette Center for Environmental Biotechnology, Arizona State University [31]. A MiSeq Illumina sequencer (Illumina Inc., Dan Diego, CA) was used for the sequencing via the chemistry version 2 (2 x 150 pair-end). Demultiplexed paired-end fastq files produced by CASAVA (Illumina) were used as inputs to QIIME2 version 2020.2 for evaluation [32,33]. Fastq files were quality filtered, trimmed, denoised, and merged with the DADA2 software package wrapped in QIIME2 [34]. Sequences were truncated at 250 basepairs due to a decline in quality of reverse reads that point. The QIIME2 feature-classifier plugin and the Naïve Bayes classifier trained on the Greengenes 13.8 99% OTU full-length sequences were used to assign taxonomy. Alpha and beta-diversity analysis was completed with the QIIME2 q2-diversity plugin at a sampling depth of 8,750. A pairwise PERMANOVA test of beta diversity significance using weighted unifrac distance was run in Qiime2 using the beta-group-significance command, and the Kruskal-Wallis test of alpha diversity significance was run in Qiime2 using the alpha-group-significance command. Raw sequences for this project are available in the NCBI SRA under the BioProject ID PRJNA631539.

3. Results and discussion

3.1 EK-Bio Treatment Outperformed Traditional Bio Method Figure 2 shows the transformation of TCE to daughter products across the Bio and EK-Bio reactors over the 11-week experiment. In the Bio reactor, products of microbial reductive dechlorination, cis-DCE and VC, appeared by week 4. Trace amounts of non-toxic ethene, 2.1-4.6 μM, appeared by week 8, but the largest concentration of daughter products remained cis-DCE and VC with average concentrations across the reactor of 86.5 μM (58.4% deviation) and 17.5 μM (52.0% deviation) respectively. Hindrance of microbial reductive dechlorination leading to cis-DCE accumulation has been reported in cultures where *Dehalococcoides* is out competed by other microbes for H_2 [28].

In the EK-Bio reactor, microbial reductive dechlorination products appeared at week 4, similar to the Bio reactor. There was also an initial spike in TCE at week 2, likely due desorption caused by electroosmosis[35]. Minimal amounts of the reductive dechlorination daughter product cis-DCE were observed in the EK-Bio reactor, but spikes of VC, 17.7 μM, were detected by week 11. Near the end of the experiment at week 9.5, ethene concentrations in the EK-Bio reactor (28.3 μM) were higher than the Bio reactor (4.6 μM). Acetylene, a TCE daughter product formed through reaction with mineral compounds or cathodic reduction, appeared in the cathode chamber of the EK-Bio reactor early on in the experiment at a concentration of 1.2 mM and reached 1.4 mM and 1.9 mM at ports 1 and 2 by week 11. By the end of the experiment acetylene concentration across the reactor averaged 824.0 μM (45.5% deviation). These results suggest both biological and chemical transformation of TCE occurred in the EK-Bio reactor as acetylene is the signature product of abiotic reaction while VC is a signature daughter product of microbial reductive dechlorination found infrequently in abiotic reactions [20].

3.2 Conditions for Microbial Reductive Dechlorination Eventually Achieved in Both Reactors Differing dechlorination results can be attributed to variations in substrate transport rates and operating conditions in each reactor. In the Bio reactor, conditions for microbial reductive dechlorination were reached at a slower pace than in the EK-Bio reactor. A negative ORP reflective of anaerobic conditions was achieved in the cathode side of the Bio reactor near the injection port by week 3, prior to injection of bioaugmentation culture, but reducing conditions were reached in the anode side only by week 9. Acetate, the fermentation product of lactate and an indication of anaerobic conditions, was not measurable in the Bio reactor until week 6. Concentrations of acetate remained low, < 13 mM, until week 10 when increased concentrations were measured in the anode and cathode chambers, 43.9 and

38.5mM respectively. While more time was required to reach reducing conditions and transport substrate, pH remained near neutral in the Bio reactor for the duration of the experiment.

Contrastingly, in the EK-Bio reactor conditions for microbial reductive dechlorination were reached quickly, though with greater challenges for pH control. A negative ORP was achieved by week 2, immediately following injection of lactate in week 1. Detection of lactate was delayed until week 8, but once measurable, was present at high concentrations, approximately 1-2mM, and evenly distributed throughout the contaminated soil. Concentrations of acetate peaked at week 10, around 60 mM. This increase in acetate, seen in both reactors at week 10, may have been due to uneven flow paths leading to areas of high concentration or to acetate produced via inorganic carbon and hydrogen via acetogenesis (Figure 4). Particularly in the EK-Bio reactor, the high relative abundance of these genera at port 4 corresponds to acetate peaks at week 10 near the anode chamber port 4. A large pH gradient developed in the EK-Bio reactor by week 2 (Figure 5) but was neutralized by slightly increasing the rate of recycle between the anode and cathode. This gradient reappeared by week 10 suggesting an even greater recycling rate or added buffer might be needed. While pH management was more difficult in the EK-Bio reactor, reducing conditions were achieved earlier due to better distribution of lactate, creating conditions that were more amenable to microbial reactions than in the Bio reactor.

3.3 Sulfate Transport and Abiotic Reactions Effect Treatment Performance Despite eventually reaching reducing conditions and diffusion of electron donor throughout the reactor, microbial reductive dechlorination stalled in the Bio reactor at cis-DCE and VC between weeks 9 and 10. This stall can be attributed to competition between Dehalococcoides and SRB due to sulfate transport limitations. As seen in Figure 6, in the Bio reactor, sulfate remained distributed throughout the soil for the duration of the experiment. By the end of the experiment, concentrations of sulfate remained over 3 mM at some locations possibly leading to competition for H2 by SRB. The competition facing *Dehalococcoides* was further confounded by with increasingly inhibitory concentrations of sulfide, already at soluble concentrations of up to 0.2mM[17]. Contrastingly, by week 3 in the EK-Bio reactor sulfate nearly disappeared except in the anode and cathode chambers. With an expected rate of electromigration of 1.2 x 10-6 m s^{-1}, sulfate would be transported the length of the reactor in 4.5 days (calculations in SI). The accumulation of sulfate in the anode and cathode chambers by week 3 is reflective of this quick migration rate. Competition for H_2 between SRB and Dehalococcoides was quickly eliminated, allowing microbial reductive dechlorination to ethene to proceed uninhibited.

The reduction of sulfate in the EK-Bio reactor may have also contributed to formation of TCE daughter product acetylene. Hydrogen sulfide generated from microbial sulfate reduction (equation 3) can react with iron oxide/hydroxide species to form elemental sulfur and iron (II) sulfide (equation 4). The iron (II) sulfide subsequently reacts with TCE to form acetylene (equation 5), as outlined in the reactions below[36]. Sulfate removal of up to 75%, along with low soluble sulfide concentrations measured (Figure 6), suggest precipitation of metal sulfides. Transformations of chlorinated ethenes via this biogeochemical pathway have been reported in lab studies and in the field through monitored natural attenuation schemes or engineered systems[36–38]. Measurements of insoluble iron species by the SEM-EDX averaged 3.8% by weight, indicating a high enough concentration to transform available TCE to acetylene.

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad (3)$$

$$2FeOOH + 2H_2S \rightarrow 2FeS + S^\circ + 4H_2O \quad (4)$$

$$4FeS + 9C_2HCl_3 + 28H_2O$$

$$\rightarrow 4Fe(OH)_3 + 4SO_4^{2-} + 9C_2H_2 + 27Cl^- + 35H^+ \quad (5)$$

Generally, the rate of microbial reductive dechlorination has been reported to be much faster than abiotic reaction with iron minerals[20]. However, the rate of abiotic reaction can be significantly increased at sites with favorable environmental conditions which increase reactant loading, as occurred in the EK-Bio reactor[20]. In addition to high concentrations of organic carbon, iron, or sulfate, the abiotic reaction rate can be accelerated with increases in pH[39]. Weerasooriya and Dharmasena [39] demonstrated a monotonic increase in reaction rate between iron (II) sulfide and TCE from 0.03 h^{-1} at pH 8 to over 0.05 h^{-1} at pH 10. While the pH spikes in the EK-Bio reactor were detrimental to microbial reductive dechlorination, they may have aided reaction rates of biogeochemical transformation. While both the EK-Bio and Bio reactors had high levels of sulfate and iron needed to generate the reactive chemical species, but quicker attainment of reducing conditions and more uniform organic carbon substrate distribution in the EK-Bio reactor may have better facilitated biogeochemical reduction of TCE.

Alternatively, acetylene can be generated through direct cathodic reduction of TCE. Cathodic reduction of chlorinated solvents has been investigated previously with Pt, Pd, iron, and graphite electrodes in closed recirculation systems [23–25]. TCE can follow several abiotic dechlorination pathways with multiple daughter products, but the appearance of acetylene indicates β-elimination was likely the mechanism. The use of a graphite electrode has been reported to lead to the by-product chloromethane, a known carcinogen, through the combination of chloride and methyl radicals created through the Kolbe reaction of acetate [25]. No chloromethane was detected in this experiment, likely as acetate was consumed by SRB or dechlorinating bacteria.

Under similar conditions in a soil free reactor with a granular graphite electrode and the application of 15 V, Al-Abed and Fang (2007) measured transformation of 76% of TCE to ethene and ethane in 25 hours. In this EK-Bio experiment, acetylene may have been the primary reaction product rather than ethene or ethane as it volatilized out of solution into the gas bag, preventing further reaction with the cathode or iron species in the soil. The early appearance of acetylene in the cathode chamber of the EK-Bio reactor and the complete absence of acetylene in the Bio reactor suggest cathodic reduction was the primary, or at least initial, abiotic transformation mechanism of TCE in this experiment[25]. The rate of electroosmosis, the primary transport mechanism for acetylene, is 2.9 x 10^{-7} m s^{-1} (calculations in SI). At this rate, which is similar to those previously reported, acetylene generated in the cathode chamber by cathodic reduction could travel to the anode chamber in approximately 16 days[40]. The

appearance of acetylene in the anode chamber at week 6 could be due to cathodic reduction in earlier weeks and subsequent electroosmostic transport throughout the experiment. Thus, the presence of acetylene throughout the reactor does not necessarily imply a production through FeS minerals. In fact, the early appearance of acetylene within the cathode chamber suggests that cathodic reduction was likely the primary, if not sole, abiotic transformation mechanism.

3.4 Microbial Community Analysis Reveals Large Shift in EK-Bio Reactor Analysis of the final resulting microbial community structures shows differences between the native microbial community, the Bio reactor samples, and the EK-Bio reactor samples. The initial soil samples are very similar to those in the Bio reactor. The samples from the EK-Bio reactor are very different from the initial soil samples (P = 0.020) or the samples from the Bio reactor (P = 0.001), especially near port 4. These data and the matrix distances can be seen in the weighted unifrac beta (between sample) diversity plot in in Figure 7A. In weighted unifrac analysis, the most abundant taxa drive differences between the distance matrices. The differences between samples from the EK-Bio reactor and others is likely due to the larger increase in fermentative and sulfate reducing bacteria, this is illustrated in more detail by our taxonomic analysis in Figure 8. Further, the microbial community appeared to be most different at port 4 of the EK-Bio reactor, which is consistent with the taxonomy data showing an increase in phylotype similar to Bacilli at this location (Figure 8).

Alpha (within sample) diversity in the Bio reactor was largely unchanged from that of the initial soil microbial community (P = 0.79; Kruskal-Wallis test), but significantly decreased in the EK-Bio reactor (P = 0.038; Kruskal-Wallis test), especially near ports 1 and 4 (Figure 7B). Average operational taxonomic units (OTUs) in the initial soil sample were 803 versus 842 in the Bio reactor and 449 in the EK-Bio reactor. This type of decrease in alpha diversity has been previously reported in the literature and attributed to secondary effects of EK like changes in pH, which is consistent with chemical data reported here in week 10 (Figure 5)[41–43]. This drop can also be linked to the enrichment and increased abundance of one microbe, in this case, phylotypes most similar to the class Clostridia.

Assessment of the microbial community members shows many similarities between the Bio and EK-Bio reactors[28]. Both reactors displayed comparable levels of the phylotype most similar to dechlorinating bacteria, though phylotypes most similar those found in the overall enrichment culture were slightly more elevated in the EK-Bio reactor. The microbial community of the enrichment culture was previously described in Delgado et al. 2014 and includes, among others, Clostridia, Bacteroidia, Anaerolineae, and Dehalococcoidetes [28]. Clostridia, Bacteroidia, and Anaerolineae are known to contain fermentative bacteria and are frequently found in the environment[44–46]. The class Clostridia is also known to contain species of SRB[47]. Phylotypes similar to the classes Clostridia, Bacteroidia, and Anaerolineae, constituted on average, 27% of the community in the Bio reactor and 43% of the community in the EK-Bio reactor (Figure 8). Phylotypes similar to the class Dehalococcoidetes, which contains the TCE dechlorinating *Dehalococcoides*, made up 0.1% or less of each community in both reactors, but were slightly more abundant in the Bio reactor. Both reactors also displayed similar levels of the phylotype most similar to the class Deltaproteobacteria, which is known to also contain SRB and iron reducing bacteria[47]. Unlike the Bio reactor, there was also a significant enrichment of phylotypes most similar to the class Bacilli near port 4 of the EK-Bio reactor. Analysis on a genus level (not shown) indicates this was most similar to the phylotype *Ammoniphilus*, an aerobic haloalkalitolerant organism[48]. Enrichment of this organism near port 4 may have been due oxygen production near the anode. The relative abundance of phylotypes most similar to genera known to have homoacetogenic metabolisms can be seen in Figure 4. Overall, these microbial community results support the chemical data which indicate microbial reductive dechlorination facilitated by a bioaugmentation culture occurred in both reactors.

4. Conclusion

Electrokinetic and traditional bioremediation approaches both resulted in transformation of TCE in a clay soil matrix with high sulfate concentrations. Microbial reductive dechlorination was the primary mechanism in the Bio reactor with a traditional bioremediation approached. These microbial reactions stalled at the end of the experiment, likely due to competition for H_2 caused by SRB or inhibitory effects of the sulfate reduction product sulfide [17]. Greater transformation of TCE occurred in the EK-Bio reactor, where acetylene was the primary daughter product, indicating the dominance of an abiotic mechanism, either biogeochemical reaction or direct cathodic reduction. The production of acetylene near the cathode during the first few weeks of experiment in the EK-Bio reactor strongly suggest that electrochemical reduction was the major mechanism of TCE reduction. The appearance of VC and ethene in the EK-Bio reactor indicates microbial reductive dechlorination occurred as well, though as a secondary transformation mechanism. Taxonomic analysis showed enrichment of phylotypes similar to those reported in the dechlorinating inoculum in each reactor, supporting the conclusion that microbial reduction dechlorination occurred in both reactors, though to different extents [28]. Results of microbial community structure analysis are very similar to previously published work which reports some decreases in alpha diversity and beta diversity along the treatment zone [41,49,50]. The results of this experiment show that the combined biotic and abiotic mechanisms of EK-Bio can result in improved remediation over traditional bioaugmentation methods. These two mechanisms can act synergistically with microbial reductive dechlorination consuming acetate to prevent electrochemical generation of the carcinogen chloromethane and with abiotic formation of acetylene from TCE acting as a fermentable substrate for microbial reactions. Results of this work demonstrate EK-BIO can be considered a feasible remedy for chlorinated solvent contaminated environments with transport limitations and geochemical challenges, thus extending much needed treatment to a great number of impacted water sources.

Conflicts of interest

There are no conflicts to declare

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Water Impact Statement

Trichloroethene (TCE) is one of the most widespread contaminants in groundwater affecting an estimated 4.5-18% of drinking water sources in the United States. Combined remediation technologies are required to address the increasingly complex sites which remain polluted. Here, we present a combined bioelectrochemical approach which improves treatment outcomes and extends applications of traditional technologies.