Effect of alternating bioremediation and electrokinetics on the remediation of \(n\)-hexadecane-contaminated soil

Sa Wang\(^1,2\), Shuhai Guo\(^2\), Fengmei Li\(^3\), Xuelian Yang\(^3\), Fei Teng\(^3\) & Jianing Wang\(^4\)

This study demonstrated the highly efficient degradation of \(n\)-hexadecane in soil, realized by alternating bioremediation and electrokinetic technologies. Using an alternating technology instead of simultaneous application prevented competition between the processes that would lower their efficiency. For the consumption of the soil dissolved organic matter (DOM) necessary for bioremediation by electrokinetics, bioremediation was performed first. Because of the utilization and loss of the DOM and water-soluble ions by the microbial and electrokinetic processes, respectively, both of them were supplemented to provide a basic carbon resource, maintain a high electrical conductivity and produce a uniform distribution of ions. The moisture and bacteria were also supplemented. The optimal DOM supplement (20.5 mg·kg\(^{-1}\) glucose; 80–90% of the total natural DOM content in the soil) was calculated to avoid competitive effects (between the DOM and \(n\)-hexadecane) and to prevent nutritional deficiency. The replenishment of the water-soluble ions maintained their content equal to their initial concentrations. The degradation rate of \(n\)-hexadecane was only 167.0 mg·kg\(^{-1}\)·d\(^{-1}\) (1.9%, w/w) for the first 9 days in the treatments with bioremediation or electrokinetics alone, but this rate was realized throughout the whole process when the two technologies were alternated, with a degradation of 78.5% ± 2.0% for the \(n\)-hexadecane after 45 days of treatment.

There has been a wealth of research into improving the remediation efficiency of organic-contaminated soils\(^1-5\). Traditional bioremediation and biological intensifying measures that have been widely used include the addition of surfactants\(^6\), exogenous nutrients\(^7\) and chemical oxidants\(^8,9\) and the use of electrokinetics\(^10\). These techniques aim to provide optimal reaction conditions. To enhance microbial remediation by increasing microbial metabolism, dissolved organic matter (DOM) is an important co-substrate and has been recognized as one of the most critical carbon sources\(^11\). Furthermore, the DOM concentration affects the quantity and degradation abilities of microbes\(^8,12\). Han et al.\(^13\) found that the addition of DOM could enhance the microbial degradation of organic contaminants in soils; similar results were shown in increases in the solubility and biodegradation of contaminants due to the exogenous application of DOM\(^14,15\). However, a competitive effect can be induced if excessive concentrations of DOM are added, which is not conductive to biodegradation\(^16-18\). Therefore, the optimum range for the DOM content should be determined.

Electrokinetic remediation is a green and powerful remediation technology that has been extensively studied for use in organic-contaminated soils, in terms of the electrochemical mechanisms of electromigration, electroosmosis and electrophoresis\(^16\). The results have shown improvements in the removal of organic contaminants from soils\(^20,21\). However, electrokinetic remediation changes the soil properties, including the soil pH, moisture and microbial biomass\(^22,23\); these effects limit the current that can be applied\(^24\), which limits the efficiency of electrokinetic remediation in long-term treatment processes\(^10\). Polarity reversal has been shown to limit the changes in soil properties\(^25,26\), but if employed, water-soluble ions need to be added to replenish their losses and improve the heterogeneous distribution of the ions in non-uniform electric fields.

\(^1\)Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China. \(^2\)University of Chinese Academy of Sciences, Beijing 100049, China. \(^3\)Shenyang University, Shenyang 110014, China. \(^4\)Institute of Biology, Shandong Academy of Sciences, Jinan 250014, China. Correspondence and requests for materials should be addressed to S.G. (email: shuhaiguo@iae.ac.cn)
Table 1. Initial characteristics of the soil used in the experiment (after being air-dried and sieved through a 2 mm mesh).

| Soil properties | Value |
|----------------|-------|
| pH             | 7.65  |
| CEC (cmol·kg⁻¹) | 32.66 |
| Organic C (OC) (g·kg⁻¹) | 10.50 |
| DOC/OC         | 0.36  |
| Soil Texture (mm, %) |       |
| <0.002         | 12.3  |
| 0.002–0.02     | 21.4  |
| 0.02–2.00      | 66.3  |

The use of electrokinetic techniques in combination with bioremediation vastly improves the efficiency over that of electrokinetic remediation alone in removing organic pollutants from soil27–30. For example, the remediation efficiency was prominently improved using electrokinetic–bioremediation (EK-Bio) in soils contaminated with petroleum and its components25,31,32. The EK-Bio degradation process is highly efficient during the initial stages of treatment owing to the stimulation of bacterial activity by the weak electric field31,32. However, the remediation rates decrease over time, as the soil DOM and inorganic ion contents change and impact both the bioremediation and electrokinetic processes. Therefore, a combined method that precludes the interference between the bioremediation and electrokinetic technologies should be developed. The purpose of this study was to combine the highly efficient degradation phases of bioremediation and electrokinetics to realize a rapid remediation process for \( n \)-hexadecane-contaminated soil.

This experiment explored a feasible method in which bioremediation and electrokinetic techniques were alternated to avoid mutual interference. The soil moisture, DOM, and water-soluble ion contents and the microbial community were supplemented to ensure that the soil micro-environment was optimal for the microbial and electrokinetic remediation processes. \( n \)-Hexadecane, a middle-chain model compound that represents aliphatic hydrocarbons, was used as the test pollutant; it is a chemically stable and persistent pollutant due to its nonpolar properties and has been similarly used in other studies33,34.

Materials and Methods

Soil and chemicals. The soil used in the present study was classified as sandy loam. It was obtained from the Institute of Applied Ecology experimental station, located in Shenyang, China. The soil, taken at 0–10 cm depth, had a slightly alkaline pH and soil organic carbon content typical of the region (Table 1). The collected soil was air-dried and sieved through a 2 mm mesh to create a consistent and homogeneous mix. To artificially contaminate the soil for the experiment, it was mixed with \( n \)-hexadecane (>98%; Sinopharm Chemical Reagent Company, Shanghai, China). The density of the \( n \)-hexadecane in the analytical grade reagent was 0.77 g·cm⁻³, and its melting point was 18.3 °C. An \( n \)-hexadecane-\( n \)-hexane solution was slowly mixed into the soil, and the soil-\( n \)-hexadecane-\( n \)-hexane mixture was agitated in a ventilation hood for two weeks to ensure uniform distribution of the \( n \)-hexadecane until the \( n \)-hexane was thoroughly evaporated. \( \text{NH}_4\text{NO}_3 \), \( (\text{NH}_4)_2\text{SO}_4 \) and \( \text{KH}_2\text{PO}_4 \) (analytical grade, Sinopharm Chemical Reagent Company, China) were also mixed into uncontaminated soil (control treatments; cEK and cBio1) to allow for observations of changes in the soil characteristics. \( \text{K}_2\text{HPO}_4 \), \( \text{MgSO}_4 \), \( \text{NaCl} \), \( \text{CaCl}_2 \) and \( \text{FeSO}_4 \) (analytical grade, Sinopharm Chemical Reagent Company, China) were prepared for the nutrient solution. Dichloromethane and acetone, used for extracting \( n \)-hexadecane from the soil, were analytical grade (Sinopharm Chemical Reagent Company, China).

Bacteria cultures. Two types of petroleum-degrading mixed bacterial culture (Culture A and Culture B) were used, depending on the treatment stage. Culture A was isolated from oil-contaminated soil adjacent to Liaohu Oil Field (Liaoning province, Northeast China), and Culture B was prepared from soil samples removed at the end of the first EK stage (EK1) in the Bio1 + EK1 + Bio2 + EK2 treatment (see ‘Experimental design’). The nutrient solution used for the bacterial cultures was maintained at pH 7.0 and consisted of \( \text{KH}_2\text{PO}_4 \) (2.75 g·L⁻¹), \( \text{K}_2\text{HPO}_4 \) (2.25 g·L⁻¹), \( \text{MgSO}_4 \) (0.2 g·L⁻¹), \( (\text{NH}_4)_2\text{SO}_4 \) (1.0 g·L⁻¹), \( \text{NaCl} \) (0.5 g·L⁻¹), \( \text{CaCl}_2 \) (0.02 g·L⁻¹), \( \text{FeSO}_4 \) (0.02 g·L⁻¹), a microelement stock solution (1 ml·L⁻¹) and petroleum (0.5 g·L⁻¹). The bacteria were cultivated on a constant temperature shaker at 30 °C and 180 rpm, and their growth was monitored by measuring the absorption of the culture at OD600 nm; they were harvested by centrifugation during their exponential phase of growth. The bacterial precipitate was resuspended in mineral media to form a bacterial suspension, and only Culture A was evenly mixed into the soil for the initial experiment.

Experimental design. Seven treatment regimes, summarized in Table 2, were performed in duplicate: controls of each technology type without contamination (cBio1 and cEK), a control with contamination but without bacteria or electrokinetic remediation (CK), a bioremediation-only treatment with Culture A (Bio1), an electrokinetic remediation only treatment (EK), a bio–electrokinetic simultaneous remediation treatment (Bio1-EK), and a treatment in which the applications of bioremediation and electrokinetic remediation were alternated (Bio1 + EK1 + Bio2 + EK2). In the treatments that used contaminated soil, it was contaminated with approximately 1% (v/w) \( n \)-hexadecane (8.9 g·kg⁻¹). The moisture content of the soil in all treatments was maintained at 20% (w/w) by adding deionized water once every 6 days during the electrokinetic treatment stages. For
cBio1 and cEK, inorganic ions were added, including NH₄NO₃, (NH₄)₂SO₄ and KH₂PO₄, to amplify the ionic changes that occurred under each treatment technology. Nutrient solution was added to all of the bioremediation and electrokinetic remediation treatments (Bio1, EK, Bio1-EK, Bio1 + EK1 + Bio2 + EK2; Table 2). At the start of the bioremediation treatments, bacteria Culture A was added to achieve an initial concentration of 4.5 × 10⁸ copies·g⁻¹ of dry soil. For the Bio1 + EK1 + Bio2 + EK2 treatment, before the ‘Bio2’ stage, the soil was thoroughly mixed and bacteria Culture B (microbial supplement), nutrient solution, and glucose (as an easily utilizable carbon source) were added to promote microbial metabolism. The added glucose compensated for the loss of the DOC, according to its consumption in the cBio1 and cEK treatments; these treatments were carried out because n-hexadecane can interfere with the extraction of the DOM. Thus, by treating uncontaminated soil, the variations in the concentration and distribution of the DOM and inorganic ions in the soil could be monitored. In the Bio1 + EK1 + Bio2 + EK2 treatment, the points of switching between the distinct treatment methods were determined by decreases in the observed degradation rates of n-hexadecane. Each experiment was conducted at room temperature (25 ± 1 °C). The completed duration for CK, Bio1, EK and Bio1-EK was 54 days; for the cBio1, cEK and Bio1 + EK1 + Bio2 + EK2 treatments it was 45 days.

Electrokinetic apparatus. The electrokinetic apparatus (Fig. 1A) consisted of a Perspex soil chamber with inner dimensions (L × W × H) of 24 × 12 × 12 cm, a power supply, two pairs of electrodes and an electrode control system. The electrodes were made of graphite, with a length of 15 cm and a diameter of 0.5 cm, and were inserted parallel to each other at either end of the test soil cell. An electric field was generated with a constant voltage gradient of 1.3 V·cm⁻¹. The polarity of the electric field was alternated, by the electrode auto-control system, every 2 hours during the electrokinetic treatment processes (Table 2).

Sample collection. In the electrokinetic and bioremediation treatments (Bio1, EK, Bio1-EK and Bio1 + EK1 + Bio2 + EK2), samples were collected every 3 days to monitor the degradation rate of n-hexadecane, and the same was done for CK. A total of 15 samples (5 × 3 samples in each chamber) were collected, and all samples were evenly mixed together before determining the n-hexadecane concentrations (Fig. 1B).

For the cBio1 and cEK tests, 15 samples (5 × 3 samples in each chamber) were gathered every 9 days. The three samples from each sampling line (Fig. 1B) were mixed uniformly to measure the water-soluble ions, according to their distance from the electrodes (Fig. 1B). A composite sample of all samples mixed together was also prepared to determine the total ion and dissolved organic matter (DOM) content. All soil samples were stored at −20 °C until analysis.
Finally, the soil electrical conductivity was measured in the EK, EK1 and EK2 samples under a soil to water ratio of 1:5 using a conductivity metre (DDS-11A, INESA, China)\(^\text{35}\), and the current in the EK tests was recorded periodically using an ammeter.

**n-Hexadecane analysis.** The n-hexadecane was extracted using dichloromethane and acetone. First, a 3.0 g freeze-dried soil sample was mixed with 30 mL of dichloromethane and acetone (1:1, v/v) in a 250 mL conical flask, and then the following extraction steps were performed three times: (1) shaking for 30 min at 180 rpm, (2) immersion in an ultrasonic bath for 10 min, and (3) centrifugation for 2 min at a speed of 8000 rpm. A total of 90 mL of dichloromethane and acetone (1:1, v/v) were used for the whole extraction. The supernatant from the extraction steps was concentrated in a pear-shaped flask and then dissolved in 5.0 mL n-hexane for quantitative analysis. A Thermo Scientific TRACE gas chromatograph equipped with a flame ionization detector (GC-FID) was used for the analysis. An HP-5 capillary column was used with highly pure nitrogen as a carrier gas at a constant flow rate of 1.0 mL·min\(^{-1}\). The temperatures of the injector and detector were 250 °C and 300 °C, respectively. The temperature rose from 60 °C to 290 °C at 15 °C min\(^{-1}\) and was maintained at 290 °C for 5 min. The concentration of n-hexadecane was calculated using an external standard method for the authentic standard substance.

**DOM and water-soluble ion analyses.** The DOM was extracted according to a method described by Kaiser et al.\(^\text{35}\), with some modifications. First, a 5.0 g freeze-dried soil sample was soaked in 25 mL distilled water. The suspension was shaken for 30 min and incubated at 20 °C overnight and then passed through 0.45 μm polysulfone membrane filters (Supor-450; Gelman Pall Sciences, Ann Arbor, MI). The concentration of total DOM in the filtrate, expressed as the dissolved organic carbon (DOC) concentration (Multi N/C 3000OC/TN), was determined. The total water-soluble ions, including K\(^+\), NH\(_4\)\(^+\), NO\(_3\)\(^-\), SO\(_4\)\(^{2-}\) and PO\(_4\)\(^{3-}\), that leached from the soil samples were measured according to Lu\(^\text{35}\), with some modifications. Freeze-dried soil (5.0 g) was immersed in 25 mL deionized water and maintained in suspension for 5 min. Then, it was left to settle, and the supernatant was filtered through a 0.45 μm Teflon filter, dried for 4 h at 105 °C, and weighed. During the drying process, 15% H\(_2\)O\(_2\) was added to thoroughly remove the organic C.

**Dehydrogenase activity and microbial enumeration assay.** The soil dehydrogenase (DHA) activity was measured by monitoring the rate of the reduction of triphenyl tetrazolium chloride (TTC) to triphenylformazan (TPF), as described by Oliveira et al.\(^\text{37}\), with some modifications. TPF was detected using a spectrophotometer (UV-2550, Shimadzu) at 485 nm after a dark incubation for 24 h and expressed in μg TPF d·g\(^{-1}\) of dry soil 24 h\(^{-1}\).

The bacterial biomass was analysed using the real-time PCR of the 16S rRNA gene; this was performed with the ABI Prism 7000 Real-Time PCR Detection System (Applied Biosystems, USA) using SYBR Premix Ex Taq II (2×) and ROX Reference Dye (50×) (Takara, China). A standard curve was produced using genomic DNA extracted from E. coli, as a template to quantify the total number of bacterial 16S rRNA gene copies. The primers used for amplification of the 16S rRNA genes were 8F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1528R (5′-ATTACCGCGGCTGCTGG-3′). The conditions for the real-time PCR were 30 s at 95 °C and then 40 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 45 s and 72 °C for 5 min.

**Statistical analysis.** All measurements were carried out a total of four times (two times per treatment), and their means and standard deviations were calculated and plotted using SigmaPlot 10.0 (Systat Software, USA). SPSS 21.0 (USA) was used to analyse the variance and perform Pearson’s rank correlation analyses.

An estimate of the expected n-hexadecane residues for the Bio1-EK treatment was calculated as the sum of the residues in the Bio1 and EK only treatments,

\[
\text{Residue}_{\text{Bio1} - \text{EK}} = \text{Residue}_{\text{Bio1}} + \text{Residue}_{\text{EK}} - 1
\]

where Residue\(_{\text{Bio1} - \text{EK}}\) is the estimated n-hexadecane residue in the Bio1-EK treatment and Residue\(_{\text{Bio1}}\) and Residue\(_{\text{EK}}\) are the real n-hexadecane residues in the Bio1 and EK only treatments, respectively.

The amount of glucose to add to the Bio1 + EK1 + Bio2 + EK2 treatment (before “Bio2”) was defined according to the equation

\[
m_{\text{initial}} = \frac{\text{DOC}_{\text{initial}} \times K - \text{DOC}_{\text{cBio}} - \text{DOC}_{\text{cEK}}}{W_{\text{c(Glu)}}}
\]

where \(m_{\text{initial}}\) is the amount of glucose needed; \(\text{DOC}_{\text{initial}}\) is the original quantity of DOC in the uncontaminated soil; \(\text{DOC}_{\text{cBio}}\) and \(\text{DOC}_{\text{cEK}}\) are the residual content of DOC after 9 and 12 days of the cBio and cEK tests, respectively; \(W_{\text{c(Glu)}}\) is the carbon content ratio of glucose, which is 40%; and K is the organic carbon ratio, accounting for the original amount according to the experimental design.

**Results**

**Analysis of bioremediation, electrokinetic and bio-electrokinetic remediation processes.**

**n-Hexadecane degradation.** According to the calculation of the residues shown in Fig. 2, the degradation extents of n-hexadecane in the Bio1 and EK treatments were 40.2% ± 2.0% and 44.8% ± 2.2%, respectively, after 54 days of remediation. Linear degradation rates occurred in both treatments during the first 9 days. Both treatments were relatively efficient at degrading n-hexadecane until 27 days had passed; after this point, the removal efficiencies remained low. The degradation rates in the Bio1-EK treatment were improved compared with those of the
Bio1 (p < 0.05, 54 days) and EK (p < 0.05, 54 days) only treatments. Obviously higher rates with both treatment technologies working together in the Bio1-EK treatment occurred during the first 12 days. Then, the degradation rate of \( n \)-hexadecane started to decrease. In total, 69.9% ± 2.2% of the compound was removed by the end of the experiment (Fig. 2).

The curve of the \( n \)-hexadecane residue in the Bio1-EK treatment agreed well with the simulation (calculated by Equation (1)) during the initial phase. However, with further incubation, the degradation extent in the Bio1-EK treatment was prominently lower than expected, and there was a period where the degradation ratio of the Bio1-EK treatment was consistently 15.1% lower than expected (p < 0.05) (Fig. 2).

Soil DOC and appropriate content. The amount of DOC decreased to 66.9% ± 1.7% of the original in the cBio treatment, a decline of 33.1% ± 2.1%, after 45 days (Fig. 3); this reflects on the importance of DOC as an easily available carbon source for soil microbial metabolism. During the first 9 days, 11.5% ± 1.5% of the DOC was consumed. The microbial biomass, estimated from the 16S rRNA gene copies, increased from the outset, corresponding to intensive DOC consumption. However, the DOC could compete with \( n \)-hexadecane as a carbon source for the microorganisms, which could have reduced the effectiveness of the bioremediation in the contaminated soil. There was a prominent decline in microbial biomass after 27 days of remediation, and after this point, the DOC content remained at 74.9% ± 3.8%, revealing that the DOC content was too low for the microorganisms. According to Fig. 3, the maintenance of the DOC content between 80% and 90% of the initial amount (marked by

---

**Figure 2.** Residues of \( n \)-hexadecane in the EK, Bio1 and Bio1-EK treatments during the 54-day experimental period. Data shown are the means ± S.D. (n = 4).

**Figure 3.** Total bacterial 16S rRNA gene copy number and DOC content (% of initial) in the cBio1 treatment during the 45-day experimental period. Data shown are the means ± S.D. (n = 4).
between them, leading to a maximally efficient remediation process. Moreover, the combination of two technologies (electrokinetics and bioremediation) might optimize each technology’s remediation efficiency and alleviate the interference between the two remediation technologies during the long-term treatment when conducted simultaneously. Hence, alternating the two technologies (electrokinetics and bioremediation) might optimize each technology’s remediation efficiency and alleviate the interference between them, leading to a maximally efficient remediation process.

**Figure 4.** Variation of the electric current (A) and soil electrical conductivity (B) over time during the EK treatment. Data shown are the means ± S.D. (n = 4).

dotted lines in Fig. 3) should be an appropriate scope to minimize the competitive effects and extend the highly efficient period of bioremediation.

The bacterial biomass monitored in the Bio1 test reached 2.6 × 10^6 copies·g^−1 soil after 42 days, compared with 4.5 × 10^6 copies·g^−1 soil initially; that is, it was 42.5% lower after 42 days (p < 0.05), corresponding to the weak biodegradation of n-hexadecane shown in Fig. 2.

**Current, electrical conductivity and water-soluble ions during the electrokinetic remediation process.** In the EK treatment, the electric current increased after water replenishment (which was done every 6 days), reached a peak and then decreased (Fig. 4A) until the water was replenished again. The maximum current was recorded during the first cycle, where it reached 82.8 mA after the third day. The increase after each water replenishment was less than that in the last cycle, and it eventually dropped to 56.3 mA. The re-injection of deionized water was periodically done to maintain a moisture content of 20% (w:w) and increase the current, which clearly happened; these results demonstrate that water replenishment is necessary to maintain a reasonable current during the EK treatments.

The variation of the soil EC in the composite samples (Fig. 4B) was induced by the applied electric field during the EK treatment. There was an obvious initial increase in EC, up to 1333 μs·cm^−1 after 6 days. Then, the EC value rapidly decreased and was held at ca. 300–350 μs·cm^−1, that is, it decreased by 73.7–77.5% (compared to its peak) after 39 days of remediation (p < 0.05). Although the water was replenished, the low maximum currents, arising from the low soil EC levels, also indicate that the effects of the electrodynamics and electrochemistry decreased after 9 days of treatment; this corresponds to the nonlinear decreases in the n-hexadecane residues during the EK treatment (Fig. 2). To restore the original electric current and sustain high soil EC levels, an ionic solution (like the nutrient solution used in this study) should be added during treatment; if this was done, the expected EC values would be higher (Fig. 4B).

There was an obvious non-uniform distribution of the water-soluble ions in the cEK treatment, with prominent aggregations located in the vicinity of both electrodes after 9 days of treatment and the completion of a whole polarity cycle (Fig. 5); there was an average of 48.5% ± 1.7% more water-soluble ions at the electrodes than in the middle regions of the electric field (p < 0.05). Furthermore, there were more ions concentrated around the initial cathode compared with the initial anode (p < 0.05). Remarkably, the average quantity of the water-soluble ions was 32.9% ± 6.3% less in 9 days treatment than in the initial sample (0 days) (scatter; Fig. 5) (p < 0.01).

**Effect of electrokinetics on bioremediation.** Even though the n-hexadecane degradation appeared to be improved in the Bio1-EK treatment compared with the Bio1 and EK treatments, the electrokinetics detrimentally affected the microorganisms in the later phases of the Bio1-EK treatment. This was demonstrated indirectly in the consumption of the DOC (the DOC content in the cEK treatment was 31.7 ± 1.4 mg·kg^−1 after 9 days of treatment, a decrease of 16.2% ± 3.7% of the natural DOC content in the soil sample (p < 0.05)) and directly in the microbial numbers and DHA levels. The number of 16S rRNA copies after 9 days of the EK treatment was just 10^6 copies·g^−1 soil because there was no addition of exogenous oil-degrading bacteria; this was 48.2% ± 2.4% less than in the natural soil sample at 0 days (p < 0.05) (Fig. 6). Similarly, a decrease of 18.8% ± 1.8% in the DHA activity was achieved after 42 days of treatment in the Bio1-EK treatment (p < 0.05).

The DHA activity of the soil samples in the Bio1-EK test decreased by 10.6 ± 0.4 μg TPF·g^−1 dry soil 24 h^−1 between 3 (maximum observed) and 42 (minimum observed) days of treatment (Fig. 7), and a highly significant positive correlation was observed between the degradation extent of n-hexadecane and the DHA activity (R = 0.587, p < 0.05, n = 16), even though part of the n-hexadecane was degraded by the electrokinetic treatment in Bio1-EK.

All of these factors indicate the nonlinear remediation rate over the Bio1-EK treatment duration (Fig. 2) and suggest that there was interference between the electrokinetic and bioremediation technologies during the long-term treatment when conducted simultaneously. Hence, alternating the two technologies (electrokinetics and bioremediation) might optimize each technology’s remediation efficiency and alleviate the interference between them, leading to a maximally efficient remediation process.
Remediation efficiency using alternating electrokinetic and bioremediation technologies.  
Assessment of the alternating pattern.  Because of the interference between the electrokinetic and bioremediation  
technologies, we investigated the application of bioremediation (Bio1) prior to the electrokinetics (EK1)  
and then prolonged the process by repeating the two treatment cycles (Bio1 + EK1 + Bio2 + EK2). During the  
treatment, the soil micro-environment was adjusted to optimize the remediation effects. In cBio1, 4.9 mg·kg⁻¹  
of DOC was consumed after 9 days of treatment, accounting for 11.5% ± 1.3% of the total DOC present (Fig. 3), and  
during the 12 days of the cEK treatment, 7.5 mg·kg⁻¹ (19.9% ± 1.6% of the total DOC) was consumed. Therefore,  
the amount of DOC consumed through Bio1 + EK1 was estimated to be 12.4 mg·kg⁻¹. To create the optimum  
DOC conditions (80–90% of original DOC; Fig. 3) for the second half of the Bio1 + EK1 + Bio2 + EK2 treat-  
ment (Bio2 + EK2), 20.5 mg·kg⁻¹ glucose (calculated by Equation (2)) was mixed into the soil (to achieve 90% of  
original DOC content) before the Bio2 stage. The content of inorganic ions dissolved in the nutrient solution was  
the same as that used in the Bio1 bacterial culture (see 'Bacteria cultures'). The soil was also supplemented with  
Culture B prior to Bio2 because of the decreases in the biomass and DHA activity of the bacteria in the cBio1 and  
Bio1 tests over time. The bacteria observed in the EK1 stage soil samples were enriched with Culture B, which  
were adapted to organic pollution, including the intermediate products of n-hexadecane degradation. Therefore,  
the microorganisms present favoured the acceleration of the bioremediation process.

n-Hexadecane degradation. The residual levels of n-hexadecane in the Bio1 + EK1 + Bio2 + EK2 treatment,  
presented in Fig. 8A, show a linear decrease in the compound over time. After 39 days, 67.7% ± 2.2% of the  
n-hexadecane was removed, an improvement of 8.7% compared to the Bio1-EK test (p < 0.05), and 78.5% ± 2.0%  

---

**Figure 5.** Variations in the distribution (column) and overall average amount (scatter) of the water-soluble ions in the cEK treatment after 9 days. Different small letters above the columns indicate significant differences among the samples (p < 0.05). Data are shown as the means ± S.D. (n = 4).

**Figure 6.** Total bacterial 16S rRNA gene copy numbers and a comparison of the total bacterial amounts at days 0 and 9 days during the EK-only treatment (p < 0.05) (Insert). Data are shown as the means ± S.D. (n = 4).
of the \(n\)-hexadecane had been degraded after 45 days of treatment. The predicted removal of \(n\)-hexadecane, according to the combined effects of the Bio1 and EK treatments (simulated curve Bio1 + EK; Fig. 8A), was the same as the observed results of the Bio1 + EK1 + Bio2 + EK2 treatment after 45 days (circle; Fig. 8A).

The degradation rates of \(n\)-hexadecane during the Bio1 and EK treatments superimposed over that observed for the Bio1 + EK1 + Bio2 + EK2 treatment (Fig. 8B) show why the degradation rates during the Bio1 + EK1 + Bio2 + EK2 test remained high. The degradation rates for the Bio1 and EK treatments were consistently high during the initial 9–12 days and then declined. During the Bio1 + EK1 + Bio2 + EK2 treatment, the degradation rates remained linear throughout because the first part of the Bio1 or EK treatment curves were captured each time the treatment technology was alternated. Therefore, a near constant degradation rate of 167.0 mg·kg\(^{-1}\)·d\(^{-1}\) \(n\)-hexadecane from the dry soil was achieved.

The estimated values of the degradation rates for each treatment process, based on the curves, were similar to the measured values listed in Table 3. It is interesting that the sum of the estimated values for the linear stages of each individual technology was almost equal to the estimated values of the Bio1 + EK1 + Bio2 + EK2 treatment, as was the observed degradation extent (Table 3). The results indicate that the EK stages in the Bio1 + EK1 + Bio2 + EK2 test (EK1 and EK2) had similar degradation efficiencies to those estimated from the Bio1 -EK treatment upon extending the linear part of the curve to 12 days, compared to the original 9 days.
Microbial biomass, DHA activity and soil electrical conductivity. The variation of the soil EC during the electrokinetic stages of the EK1 + Bio1 + Bio2 + EK2 treatment shows that relatively high EC levels were maintained, as a result of the replenishment of the inorganic ions (Fig. 9). Compared with the soil EC during the EK treatment, which was relatively low after 9 days of treatment (Fig. 4B), the soils during the EK2 stage of the EK1 + Bio1 + Bio2 + EK2 treatment possessed EC values in the range of 751–1440 μs·cm⁻¹. The EC met, and even exceeded, the value expected based on the inorganic ion supplement (dotted lines in Fig. 4B).

The bacterial biomass fluctuated during the Bio1 + EK1 + Bio2 + EK2 treatment (Fig. 6). A decrease in the copy number occurred from the outset of the Bio1 stage. The numbers started to increase after 6 days until the end of the experiment. The gene copy number prominently increased at 21 days, after the addition of Culture B, containing bacteria cultured from the final soil sample taken during the EK1 stage. The numbers then peaked at 10⁹ copies·g⁻¹ soil after 42 days. The high microbe number ensured, to a certain extent, efficient microbial degradation during the long-term process.

The DHA activity of the soil samples in the Bio1 + EK1 + Bio2 + EK2 treatment remained high compared to that in the Bio1–EK treatment, especially during the EK1 stage and even more so during the Bio2 and EK2 stages (Fig. 7). The maximum DHA activity achieved was 31.7 ± 0.5 μg TPF·g⁻¹ dry soil 24 h⁻¹, and 22.5 ± 0.4 μg TPF·g⁻¹ dry soil 24 h⁻¹ of DHA activity was observed at the end of the test. Because of the DOC, inorganic ion and bacteria additions, the Bio1 + EK1 + Bio2 + EK2 treatment had a higher DHA activity than the Bio1–EK treatment (p < 0.05), except for during the Bio1 stage.

Discussion

The linear degradation of n-hexadecane observed throughout the Bio1 + EK1 + Bio2 + EK2 treatment could clearly be attributed to the use of alternating bioremediation and electrokinetic technologies. The synergistic effect of bioremediation and electrokinetics during simultaneous application (e.g., Bio1–EK) has been demonstrated
before \(^{32}\) and was observed here in the accelerated degradation of \(n\)-hexadecane during days 27–30 in the Bio1-EK treatment. However, the high remediation rates in the Bio1–EK treatment were not maintained. This was mainly because of the changes in the soil’s micro-environment \(^{38}\). During the bioremediation process, the easily utilizable carbon, such as DOM, was consumed from the soil micro-environment. Langwaldt et al. \(^{39}\) indicated that the labile part of the DOC, serving as a secondary carbon resource in the presence of chlorophenol contamination, helped to sustain the growth of bacteria. In another study, a marked increase in the soil microbial biomass was stimulated by the input of DOC substrates \(^{40}\). High organic matter (OM) content is typically associated with high microbial numbers \(^{41,42}\) and conversely, low levels of OM and low microbial numbers co-occur in subsurface soils \(^{43}\). These studies indicated that DOC functioned as an energy source that supported the microbial biomass in soils. With a similar conclusion, in this study, 11.5% \(\pm\) 1.5% of the total DOC content was consumed, corresponding to the sharp rise in bacterial number during the first 9 days of testing. To extend the high bioremediation rates, the maintenance of the DOC, by adding an exogenous carbon source such as glucose, was necessary. Maintaining the organic carbon content at the natural levels in this study avoided the excessive excitation of microorganisms to utilize the exogenous carbon instead of the organic pollutant; we found that keeping 90% of the overall organic carbon in the soil allowed for \(n\)-hexadecane metabolism by the microorganisms, saved costs and maintained a near-original soil micro-environment.

Because of the interference of \(n\)-hexadecane in extracting DOC, the DOC consumption levels in the cBio1 and cEK treatments were used to estimate the quantity lost during the Bio1 and EK1 phases of the Bio1 + EK1 + Bio2 + EK2 treatment. The number of bacteria in the cBio treatment was higher than that in the Bio1 + EK1 stages of the Bio1 + EK1 + Bio2 + EK2 treatment after 18 days of testing, even though there was another carbon source (\(n\)-hexadecane) that could be metabolized in the soils of the latter treatment. This means that the sum of the EK1 consumptions in cBio1 and cEK stages was likely more than that in the Bio1 + EK1 stage, and the quantity of DOC needed for the subsequent Bio2 + EK2 stages, as estimated from the cBio1 and cEK treatments, was likely appropriate. The higher microbial biomass and soil EC during the Bio2 + EK2 stages proved that the solution added before the Bio2 phase was appropriate to achieve the consistently high remediation efficiency observed during the Bio1 + EK1 + Bio2 + EK2 treatment.

Furthermore, the activity of the soil enzymes that are mainly responsible for pollutant degradation could be associated with the presence of DOM. Zhan et al. \(^{44}\) reported that DOM might counteract the inhibition on the soil enzyme activities induced by polycyclic aromatic hydrocarbons. The activity of dehydrogenase, a typical endoenzyme that can catalyse the dehydrogenation of organic compounds, correlated well with the soil micro-biomass (\(R = 0.9675\)) \(^{45}\), which was induced by the abundance of DOC. Thus, the \(n\)-hexadecane removal by bioremediation probably decreased with the consumption of DOM, implying the necessity of the organic carbon supplementation.

An analogous conclusion was drawn for the electrokinetic process. The primary mechanism of direct hydrophobic organic compound (HOCs) degradation was electrochemical oxidation, the effect of which was dependent on the strength of the electric current \(^{46-48}\). In the EK treatment, a significant positive correlation between the maximum electric current and the daily average removal efficiency of \(n\)-hexadecane was observed (\(R = 0.8800\), \(p < 0.01\), \(n = 9\)). There have been similar reports on the removal of petroleum \(^{49}\), pyrene \(^{50}\) and cypermethrin pesticide \(^{51}\) by electrokinetic remediation technologies. The decline in the degradation rate with the decrease of the current occurred (due to the higher electrical resistance) because (1) the lower moisture content and lower availability of transferable ions led to weakened electromigration rates of the ions and (2) there was a lower soil EC. These changes attenuated the electrochemical oxidation efficiency. Some of the amendments implemented recovered the deficiencies and maintained a high oxidation ability in the Bio1 + EK1 + Bio2 + EK2 treatment. An obvious increase in the electric current occurred after water replenishment, which was attributed to the desorption of inorganic ions by electrochemical oxidation \(^{24}\) and the high concentrations of \(H^+\) and \(OH^-\), produced by the electrode reactions of water \(^{51}\). In a separate study, an increase in the electrolyte concentration, through the addition of a nutrient solution, resulted in a rise in the electrical conductivity and current, with the same conclusion \(^{52}\). In this study, the regulation of the water and inorganic ion contents helped to maintain the high degradation rates observed during the EK stages of the Bio1 + EK1 + Bio2 + EK2 treatment.

According to this study, the total water-soluble ion level in the soil was the crucial factor for both remediation technologies. Generally, polarity reversal has been shown to promote a relatively even distribution of ions \(^{53,54}\). However, in this study, the distribution of water-soluble ions was heterogeneous after a complete cycle (4 h) in a non-uniform field formed with columnar electrodes. The highest concentration of ions occurred around the electrodes, with the maximum field intensity, and the ion concentrations decreased with the decrease in the field intensity, positioned between the homopolar electrodes and in the centre of the field. In addition, smaller amounts of cations precipitated under alkaline conditions near the cathode compared to the number of anions around the anode. Similar results, showing that an acidic pH favoured the migration of ions and hindered their precipitation (especially for metals), have been previously reported \(^{43,44}\). Furthermore, oxidation–reduction reactions would have occurred around the electrodes \(^{19}\). In addition to this, once the ions had precipitated or been adsorbed by the SOM, it would then take some time for the ions to dissolve again once the polarity was reversed and acidic conditions were created. This process produced a time lag that shortened the net migration duration and caused asymmetric migration, leading to the non-uniform distribution of ions. This was one of the underlying causes of the gradual weakening observed in the electric current over time and might compromise the sustainability and efficiency of the electrokinetic remediation technologies. The results prove that supplementing the soil with inorganic ions is essential to creating the ideal EK remediation conditions.

The order of the alternating Bio and EK treatments was restricted because of the impact of the two technologies on the soil properties, which were supposed to be favourable for the next remediation stage. As discussed above, the soil DOM not only affects bioremediation but also is a key factor to be considered in the alternating bioremediation and electrokinetic treatment. As the easily assimilated component of SOM, the DOM was the
principal or underlying source of nutrients and energy with the maximum bioavailability to soil microorganisms. Previous research has indicated that DOC can combine with hydrophobic organic compounds and thus make the bioavailability of contaminants increase in the aqueous phase. However, electrochemical oxidation did harm the DOM in an effective mass transfer system (using electrodialysis) and counteracted the benefits of the DOM. Some studies have observed an increase in the DOC, to some extent, after treatment with electrokinetics; however, the increase was achieved under acid soil conditions, as opposed to the alkaline soil in this experiment. Thus, employing electrokinetic treatment before bioremediation would have adversely affected the overall degradation efficiency of the alternating treatment.

For the microbial viability, the alternating pattern avoided the effects of electricity and n-hexadecane in combination on the microorganisms, providing them with more time to adapt to the extreme environment. Due to their source, from soil contaminated with petroleum, the microorganisms could maintain an ideal activity and viability while exposed to the pollutant for some time. Then, before the microbial metabolic activity fell, the use of a weak electric field caused both electrochemical oxidation and microbial stimulation. For the latter, there was an obvious increase in the cell density during the EK1 process, which was a little more than that observed in the Bio1-EK treatment after 18 days. The data indicated a promoting effect of the electrical current on microbial reproduction, in accordance with Li et al., and a stronger resistance due to adaptation to n-hexadecane and environmental stress. However, the indigenous bacteria were adversely affected and decreased during the initial phase, over 9 days, in the EK-only treatment (Fig. 6); that was another reason why the EK stage was applied after the Bio1 stage.

When the bacteria in Culture B were added for Bio2, the cell density increased sharply, and during EK2, the stimulatory effect from the electricity was again observed. It is noteworthy that the bacteria in Culture B were cultured from soil sampled at the end of EK1. Therefore, the n-hexadecane-degrading bacteria present in Bio2 had already acclimatized to the soil conditions but had been in a poor physiological state because of the environmental pressures. The intermediate products of n-alkanes have previously been shown to serve as a carbon and energy source for alkane-utilizing bacteria, creating an environment conducive to microbial survival. This was the advantage of conducting the second bioremediation phase, as reflected in the quantity of bacteria during the Bio2 and EK2 stages.

Given the costs of the remediation technologies, alternating the technologies would reduce the energy consumption by half compared to applying the EK-only treatment for the same duration. Shortening the EK period also saves on electrode costs.

Conclusion

The DOM content (mass ratio of 80–90% of original) is critical to optimize the bioremediation of alkane-contaminated soil, reducing the impacts of both competitive effects and nutritional deficiency. The water-soluble ions were positively correlated with the soil electrical conductivity. Supplementation maintained the EC value of 1300–1400 μs·cm⁻¹ to stabilize the electrokinetic efficiency. Through the regulation of the DOC and water-soluble ion contents, the integration of bioremediation and electrokinetics combined four efficient remediation processes. The kinetic curves of n-hexadecane degradation approximate straight lines, and a uniform degradation rate of 167.0 mg·kg⁻¹·d⁻¹ of n-hexadecane was achieved throughout the whole process. The alternating technology ameliorated the adverse effects of the current electrokinetic-bioremediation treatment to maximize the extent and further improved the remediation efficiency of n-hexadecane-contaminated soil.

References

1. Falciglia, P. P., Giustra, M. G. & Vagliasindi, F. G. Low-temperature thermal desorption of diesel polluted soil: influence of temperature and soil content on contaminant removal kinetics. Journal of hazardous materials 185, 392–400 (2011).
2. Pham, T. D., Shrestha, R. A., Virkutyte, J. & Sillanpää, M. Combined ultrasonication and electrokinetic remediation for persistent organic removal from contaminated kaolin. Electrochimica Acta 54, 1403–1407 (2009).
3. Zhou, W. & Zhu, L. Enhanced soil flushing of phenanthrene by anionic-nonionic mixed surfactant. Water research 42, 101–108 (2008).
4. Do, S. H., Jo, J. H., Jo, Y. H., Lee, H. K. & Kong, S. H. Application of a peroxymonosulfate/cobalt (PMS/Co(II)) system to treat diesel-contaminated soil. Chemosphere 77, 1127–1131 (2009).
5. Acar, Y. B. & Alshawabkeh, A. N. Principles of electrokinetic remediation. Environ. Sci. Technol. 27, 2638–2647 (1993).
6. Bardil, L. et al. Cyclodextrin-enhanced in situ bioremediation of polycyclic aromatic hydrocarbons-contaminated soils and plant uptake. Journal of Inclusion Phenomena and Macrocyclic Chemistry 57, 439–444 (2007).
7. Suja, E. et al. Effects of local microbial bioaugmentation and biostimulation on the bioremediation of total petroleum hydrocarbons (TPH) in crude oil contaminated soil based on laboratory and field observations. International Biodeterioration & Biodegradation 90, 115–122 (2014).
8. Sutton, N. B., Grotenhuis, T. & Rijnaarts, H. H. Impact of organic carbon and nutrients mobilized during chemical oxidation on subsequent bioremediation of a diesel-contaminated soil. Chemosphere 97, 64–70 (2014).
9. Cajal-Marinos, P., Reich, O., Moby, A. & Tuilkman, T. Treatment of composted soils contaminated with petroleum hydrocarbons using chemical oxidation followed by enhanced aerobic bioremediation. Journal of Advanced Oxidation Technologies 15, 217–222 (2012).
10. Harbottle, M. J., Lear, G., Sills, G. C. & Thompson, I. P. Enhanced biodegradation of pentachlorophenol in unsaturated soil using reversed field electrokinetics. Journal of environmental management 90, 1893–1900 (2009).
11. Yu, H., Huang, G. H., Xiao, H., Wang, L. & Chen, W. Combined effects of DOM and biosurfactant enhanced biodegradation of polycyclic aromatic hydrocarbons (PAHs) in soil-water systems. Environmental science and pollution research international 21, 10536–10549 (2014).
12. Haidar, M., Drany, A., Sires, J., Oturan, N. & Oturan, M. A. Electrochemical degradation of the antibiotic sulfachloropyridazine by hydroxyl radicals generated at a BDD anode. Chemosphere 91, 1304–1309 (2013).
13. Han, X. M., Liu, Y. R., Zhang, L. M. & He, J. Z. Insight into the modulation of dissolved organic matter on microbial remediation of PAH-contaminated soils. Microbial ecology 70, 400–410 (2015).
14. Cheng, K. Y. & Wong, J. W. Combined effect of nonionic surfactant Tween 80 and DOM on the behaviors of PAHs in soil–water system. Chemosphere 62, 1907–1916 (2006).
15. Kobayashi, T., Murai, Y., Tatsunami, K. & limura, Y. Biodegradation of polycyclic aromatic hydrocarbons by Sphingomonas sp. enhanced by water-extractable organic matter from manure compost. The Science of the total environment 407, 5805–5810 (2009).
16. Kim, M. H. & Hao, O. J. Cometabolic degradation of chlorophenols by Acinetobacter species. Water research 33, 562–574 (1999).
17. Luo, W., Zhao, Y., Ding, H., Lin, X. & Zheng, H. Co-metabolic degradation of benzenfuran-methyl in laboratory conditions. Journal of hazardous materials 158, 208–214 (2008).
18. Schafer, A. & Bouwer, E. J. Toluidene induced cometabolism of cis-1,2-dichloroethylene and vinyl chloride under conditions expected downstream of a permeable Fe(0) barrier. Water research 34, 3391–3399 (2000).
19. Acat, Y. B. et al. Electrokinetic remediation—basics and technology status. Journal of hazardous materials 40, 117–137 (1995).
20. Pazos, M., Rosales, E., Alcantara, T., Gomez, J. & Sanromán, M. A. Decontamination of soils containing PAs by electroremediation: a review. Journal of hazardous materials 177, 1–11 (2010).
21. Saichek, R. E. & Reddy, K. R. Effect of pH control at the anode for the electrokinetic removal of phenanthrene from kaolinite soil. Chemosphere 51, 273–287 (2003).
22. Li, F., Guo, S. & Hartog, N. Electrokinetics-enhanced biodegradation of heavy poly cyclic aromatic hydrocarbons in soil around iron and steel industries. Electrochimica Acta 85, 228–234 (2012).
23. DeFlaun, M. F. & Condee, C. W. Electrokinetic transport of bacteria. Journal of hazardous materials 55, 263–277 (1997).
24. Alshawabkeh, A. N., Sheahan, T. C. & Wu, X. Coupling of electrochemical and mechanical processes in soils under DC fields. Mechanisms of Materials 36, 453–465 (2004).
25. Huang, D., Guo, S., Li, T. & Wu, B. Coupling interactions between electrokinetics and bioremediation for pyrene removal from soil under polarity reversal conditions. CLEAN-Sol. Air, Water 41, 383–389 (2013).
26. Li, T., Guo, S., Wu, B., Zhang, L. & Gao, Y. Effect of polarity-reversal and electrical intensity on the oil removal from soil. Journal of Chemical Technology & Biotechnology 90, 441–448 (2015).
27. Niqui-Arroyo, J. L., Bueno-Montes, M., Posada-Baquiero, R. & Ortega-Calvo, J. J. Electrokinetic enhancement of phenanthrene biodegradation in creosote-polluted clay soil. Environmental pollution 142, 326–332 (2006).
28. Wick, L. Y., Shi, L. & Hams, H. Electro-bioremediation of hydrophobic organic soil-contaminants: A review of fundamental interactions. Electrochimica Acta 52, 3441–3448 (2007).
29. Li, T., Guo, S., Wu, B., Li, F. & Niu, Z. Effect of electric intensity on the microbial degradation of petroleum pollutants in soil. Journal of Environmental Sciences 22, 1381–1386 (2010).
30. Gill, R. T., Harbottle, M. J., Smith, J. W. & Thornton, S. F. Electrokinetic-enhanced bioremediation of organic contaminants: a review of processes and environmental applications. Chemosphere 107, 31–42 (2014).
31. Yuan, Y., Guo, S. H., Li, F. M. & Li, T. T. Effect of an electric field on n-hexadecane microbial degradation in contaminated soil. International Biodeterioration & Biodegradation 77, 78–84 (2013).
32. Guo, S. et al. Synergistic effects of bioremediation and electrokinetics in the remediation of petroleum-contaminated soil. Chemosphere 109, 226–233 (2014).
33. Stroud, J. L., Paton, G. I. & Semple, K. T. Linking chemical extraction to microbial degradation of 14C-hexadecane in soil. Geoderma 156, 474–481 (2008).
34. Partovinia, A., Naaimpoor, F. & Hejazi, P. Carbon content reduction in a model reluctant clayey soil: slurry phase n-hexadecane bioremediation. Journal of hazardous materials 181, 133–139 (2010).
35. Lu, R. K. Soil Agricultural Chemical Analysis Method (ed Lu, R. K.) (China Agricultural Science and Technology Press, Beijing, 2000).
36. Kaiser, K., Kuenpophann, M. & Zeč, W. Sorption of dissolved organic carbon in soils: effects of soil sample storage, soil–to–solution ratio, and temperature. Geoderma 99, 317–328 (2001).
37. Oliveira, A. P., Pampulha, M. E. & Bennett, J. P. A two-year field study with transgenic Bacillus thuringiensis maize: effects on soil microorganisms. The Science of the total environment 405, 351–357 (2008).
38. Baraud, F., Fourcade, M. C., Tellier, S. & Astruc, M. Modelling of decontamination rate in an electrokinetic soil processing. International Journal of Environmental Analytical Chemistry 68, 103–121 (1997).
39. Langwaldt, J. H., Munster, U. & Puhakka, J. A. Characterization and microbial utilization of dissolved organic carbon in groundwater contaminated with chlorophenols. Chemosphere 59, 983–996 (2005).
40. Tan, B., Wu, F. Z., Yang, W. Q. & He, X. H. Snow removal alters soil microbial biomass and enzyme activity in a Tibetan alpine forest. Applied Soil Ecology 76, 34–41 (2014).
41. Boopathy, R. Factors limiting bioremediation technologies. Bioresource technology 74, 63–67 (2000).
42. Anesio, A. M., Hollas, C., Graneli, W. & Laybourn-Parry, J. Influence of humic substances on bacterial and viral dynamics in freshwater. Applied and environmental microbiology 70, 4848–4854 (2004).
43. Stoner, D. L. Biotechnology for the Treatment of Hazardous Waste (CRC Press, USA, 1994).
44. Zhan, X., Wu, W., Zhou, L., Liang, J. & Jiang, T. Interactive effect of dissolved organic matter and phenanthrene on soil enzymatic activities. Journal of Environmental Sciences 22, 607–614 (2010).
45. Xie, X. M. et al. Influence of root-exudates concentration on pyrene degradation and soil microbial characteristics in pyrene contaminated soil. Chemosphere 88, 1190–1195 (2012).
46. Hamed, J., Abar, Y. B. & Gale, R. J. Pb(II) removal from kaolinite by electrokinetics. J. Geotech. Eng. 117, 241–271 (1991).
47. Ang, G. C. C. & Long, Y. W. Removal and degradation of phenol in a saturated flow by electrokinetics. Soil Agricultural Chemical Analysis Method 52, 259–271 (1999).
48. Kitaz, B., Xuhui, M., Ciblak, A. & Alshawabkeh, A. N. GeoCongress 2012 State of the Art and Practice in Geotechnical Engineering (eds Hryciw, R. D., Athanasopoulos-Zekkos, A. & Yeisler, N.) 4348–4357 (2012).
49. Xu, S., Guo, S., Wu, B., Li, F. & Li, T. An assessment of the effectiveness and impact of electrokinetic remediation for pyrene-contaminated soil. J Environ Sci (China) 26, 2290–2297 (2014).
50. Bouya, H. et al. Electrooxidation of cypermethrin pesticide: A comparative study of SnO2 and boron doped diamond anodes. Journal of Chemical & Pharmaceutical Research 47, 3468–3477 (2012).
51. Yeung, A. T. Contaminant extractability by electrokinetics. Environmental Engineering Science 23, 202–224 (2006).
52. Kim, S. S. & Han, S. J. Application of an enhanced electrokinetic ion injection system to bioremediation. Water Air Soil Pollut. 146, 365–377 (2003).
53. Kim, J. H., Han, S. J., Kim, S. S. & Yang, J. W. Effect of soil chemical properties on the remediation of phenanthrene-contaminated soil by electrokinetic-Fenton process. Chemosphere 63, 1667–1676 (2006).
54. Lynch, R. J., Muntoni, A., Ruggeri, R. & Winfield, K. C. Preliminary tests of an electrokinetic barrier to prevent heavy metal pollution of soils. Electrochimica Acta 52, 3432–3440 (2007).
55. Pengerud, A., Johnsen, L. K., Mulder, J. & Strand, L. T. Potential adsorption of dissolved organic matter in poorly podzolised, high-latitude soils. Geoderma 226–227, 39–46 (2014).
56. Ren, L. L., Ling, W. T., Ni, H. W. & Gao, Y. Z. Effect of artificial root exudates on the sorption of phenanthrene in soils. China Environ. Sci. 30, 128–132 (2010).
57. Cang, L., Zhou, D. M., Wang, Q. Y. & Wu, D. Y. Effects of electrokinetic treatment of a heavy metal contaminated soil on soil enzyme activities. Journal of hazardous materials 172, 1602–1607 (2009).
58. Li, X. et al. Direct current stimulation of Thiobacillus ferrooxidans bacterial metabolism in a bioelectrical reactor without cation-specific membrane. Bioresource technology 101, 6035–6038 (2010).
59. Radwan, S. S. & Sorkhoh, N. A. Lipids of n-alkane-utilizing microorganisms and their application potential. Advances in Applied Microbiology 39, 29–90 (1993).
Acknowledgements
This work was supported by the Water Pollution Control and Management Key Project of Science and Technology of China (No. 2013ZX07202-007) and The National High Technology Research and Development Program (“863” Program) of China (No. 2013AA06A210).

Author Contributions
S.H.G. designed the study. S.W. and F.T. conducted the experiment. S.H.G., F.M.L., X.L.Y. and S.W. analyzed the data. S.H.G. and S.W. wrote the manuscript. All authors reviewed the manuscript. The help of the manuscript revision during the process of modification was from J.N.W.

Additional Information
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Wang, S. et al. Effect of alternating bioremediation and electrokinetics on the remediation of n-hexadecane-contaminated soil. Sci. Rep. 6, 23833; doi: 10.1038/srep23833 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/