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Electrokinetic-driven bioremediation of weathered hydrocarbon-contaminated clay: Identifying the optimal voltage threshol

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ABSTRACT

Weathered hydrocarbons in clay soils pose a persistent environmental hazard due to their low mobility and strong sorption to aged organic matter, resulting in limited bioavailability and complicating remediation efforts. Electrokinetic-driven bioremediation offers a promising solution, yet the optimal voltage for enhancing the bioavailability of weathered hydrocarbons, particularly under short duration, energy efficient operation and in the presence of synthetic surfactants such as Methyl Ester Sulfonate (MES) remains unclear. This study applied a two-stage treatment consisting of electrokinetic remediation (EKR) during 48 hours followed by 33 days of bioremediation. Three laboratory reactors were operated at different voltage gradients (17 V (1 V/cm), 34 V (2 V/cm), and 42.5 V (2.5 V/cm)). Total petroleum hydrocarbon (TPH) removal and changes in hydrocarbon degrading bacterial populations were evaluated. The 2 V/cm reactor treatments achieved the highest TPH removal efficiency (90.16%), outperforming the 1 V/cm (80.38%) and 2.5 V/cm (62.31%) treatments. Reduced performance at 2.5 V/cm was attributed to electrode polarization and microbial inhibition, highlighting a critical operational threshold. Statistical analysis showed that bioelectrokinetic remediation (BIO-EKR) had a significant effect on TPH removal, $p < 0.001$ and microbial population, $p = 0.002$. Voltage significantly affected TPH removal $p = 0.042$ but did not affect microbial population $p = 0.520$. No significant interaction was observed between voltage and remediation time. These findings demonstrate that moderate voltage, short duration electrokinetic pretreatment can substantially enhance bioremediation efficiency while reducing energy demand, providing an effective strategy for remediating weathered hydrocarbon contamination in low-permeability soils.

Keywords: bioremediation, clay soil, electrokinetic remediation, weathered hydrocarbons, voltage gradient.

INTRODUCTION

In recent decades, petroleum hydrocarbon spills in soils and aquatic systems have become a critical environmental concern. Accidental leaks in the exploration, extraction, production, and transportation can lead to oil spills (Abbaspour et al., 2020; Cabral et al., 2022). Petroleum oil can contaminate soil and groundwater, which serves as the primary source for clean water requirements. Hydrocarbon compounds are one type of oil pollutant that is difficult to break down. When these chemicals pollute the soil's surface, they can evaporate, be carried away by rainfall, and then penetrate the soil where they can deposit as poisonous substances that can upset the water cycle and soil (Helmy and Kardena, 2024; Polyak et al., 2018). Contaminated soils resulting from oil spills are particularly challenging to remediate because petroleum hydrocarbons are hydrophobic, persistent, and poorly bioavailable to microorganisms, thereby resisting natural biodegradation processes (Sui et al., 2021; Yuan et al., 2023).

Bioremediation is commonly used to remediate contaminated soil by petroleum hydrocarbon. This approach utilizes the metabolic activity of microorganisms to convert hydrocarbons into simpler, less toxic compounds (Al-Hawash et al., 2018; Nwankwegu et al., 2016). In soil, petroleum hydrocarbons are generally biodegradable because hydrocarbon-degrading microbial consortia are present and typically associated with soil particles within the soil matrix (Ławniczak et al., 2020; Prakash et al., 2021). Indigenous microorganisms in contaminated soils play a role in hydrocarbon degradation (Feng et al., 2021; Lan et al., 2023). Several factors inhibit bioremediation of petroleum hydrocarbons in soil, including limited bioavailability or bioaccessibility of contaminants, inadequate nutrients, insufficient electron acceptors, and a scarcity of degrading microbes, which often hinder biodegradation, especially in clay soils with low permeability (Patowary et al., 2018; Ramadass et al., 2018).

Clay-rich soils contaminated with weathered petroleum hydrocarbons present one of the most persistent challenges in environmental remediation (Abbaspour et al., 2020; Cabral et al., 2022). Long-term exposure to atmospheric oxidation, evaporation, and microbial transformation causes hydrocarbons to become strongly adsorbed onto clay minerals and aged organic matter, significantly reducing their mobility and bioavailability. Enhancing bioavailability is therefore essential for improving the effectiveness of bioremediation. Electrokinetic remediation (EKR) is a technique that can be integrated with bioremediation to enhance the bioavailability of petroleum hydrocarbons (Cappello et al., 2019; Chen et al., 2021). This study identifies as being decoupled by Methyl Ester Sulfonate (MES) assisted electrokinetic bioremediation as a new approach to separating transport constraints and bioavailability, which have long hampered the effectiveness of weathered hydrocarbon remediation in low-permeability clay soils.

EKR techniques are carried out by passing an electric current through contaminated soil. The resulting electric field initiates multiple pollutant transport mechanisms. Electromigration occurs when ions migrate toward the opposing electrode. Electrophoresis involves the movement of charged particles, such as bacteria. Electro-osmosis refers to the movement of water (Hahladakis et al., 2014; Méndez et al., 2012). These processes are controlled by intricate mass transport phenomena and electrochemical mechanisms. Recent studies indicate that electrokinetic remediation techniques are effective in removing various contaminants from soils with low permeability (Fdez-Sanromán et al., 2021; Ossai et al., 2020). The efficiency of electrokinetic remediation is significantly affected by the applied voltage gradient, as it determines the electric field strength and directly influences microbial activity (Fan et al., 2020; Brian Gidudu and Chirwa, 2020a).

Electrokinetic-driven bioremediation is a promising approach for treating clay soils contaminated with weathered petroleum hydrocarbons. Finding the right voltage is important to balance removal efficiency, microbial activity, and energy use. Energy consumption is another major limitation of EKR, to address this, the current study implemented a shortened EKR operating period of 48 hours, followed by 33 days of bioremediation. This strategy aims to lower energy demand while maintaining the initial benefits of EKR in enhancing contaminant mobility and bioavailability. Furthermore, this study establishes MES-assisted electrokinetic bioremediation as a novel strategy to decouple bioavailability and transport limitations, which have long hindered the remediation efficiency of weathered hydrocarbons in low-permeability clay soils. MES a synthetic surfactant has not been previously investigated as an enhancing agent in EKR or BIO-EKR systems for remediation of petroleum weather hydrocarbon-contaminated clay soils. Moreover, its dual role as a desorption agent and a potential carbon source makes MES a promising enhancer for the treatment of clay soils long-contaminated with petroleum hydrocarbons.

This study investigates the effect of varying voltage levels on the bioavailability of weathered petroleum hydrocarbons, as determined by total petroleum hydrocarbon (TPH) reduction and bacterial population growth. The two-stage remediation approach, consisting of short duration electrokinetic remediation (EKR) for 48 hours followed by bioremediation for 33 days, is anticipated to enhance weathered hydrocarbon bioavailability in clay rich soils. Integrating electrokinetic remediation with bioremediation methods shows potential to effectively address clay contamination from long-contaminated oil spills or weathered hydrocarbons around the oil mining area of Cepu, Central Java, Indonesia.

MATERIAL AND METHODS

Soil sample preparation

A soil sample was collected from Cepu, Central Java, Indonesia, consisting of 49.31% clay. It exhibited a porosity of 0.42 and a moisture content of 26.96%. The initial TPH content ranged from 2–4% (w/w). The soil samples were previously crushed using a Retsch mortal grinder RM200 and sieved using an ASTM standard test sieve size 8 (2.36 mm aperture) to remove large coarse materials that could cause technical problems during the remediation process. Figure 1 presents the soil sample preparation before remediation process.

Figure 1. Soil sample preparation for remediation: (a) soil sample, (b) mortal grinder RM200, (c) sieve shaker

Experimental setup

The electrokinetic remediation reactor was made of acrylic glass and consisted of three compartments (Figure 2a) : a central compartment (17 × 10 × 12 cm) containing the contaminated soil, and two adjacent electrode compartments (6 × 10 × 12 cm). The carbon electrodes were connected to a direct current power supply to apply voltage gradients of 17 V (1 V/cm), 34 V (2 V/cm), and 42.5 V (2.5 V/cm). Tap water and a methyl ester sulfonate (MES) surfactant solution were used as electrolytes in the anode compartment at a concentration of 28 g/L. The MES solution was prepared by dissolving the appropriate amount of MES in tap water to obtain the final concentration of 28 g/L. The pH of the solution was adjusted to 7.0 ± 0.2 using 0.1 M NaOH or HCl. Figure 2b presents the experimental configuration of the electrokinetic system.

Remediation procedure

The remediation was conducted in two stages: (1) electrokinetic remediation and (2) subsequent bioremediation. The electrokinetic phase was operated for 48 hours, after which the soil was subjected to bioremediation for 33 days under ambient laboratory conditions. The incubation was carried out at 28 ± 2 °C. The bioremediation stage was conducted as a static incubation without active aeration or moisture adjustment. This was to simulate a natural attenuation scenario in clay soil where oxygen diffusion and water infiltration are limited. Indigenous microbial consortia stimulated by MES without external nutrient addition.

Samples were collected at three positions along the direction of the electric field (Figure 3): near the anode (A), central zone (B), and near the cathode (C). Additionally, samples were obtained

(a)

(b)

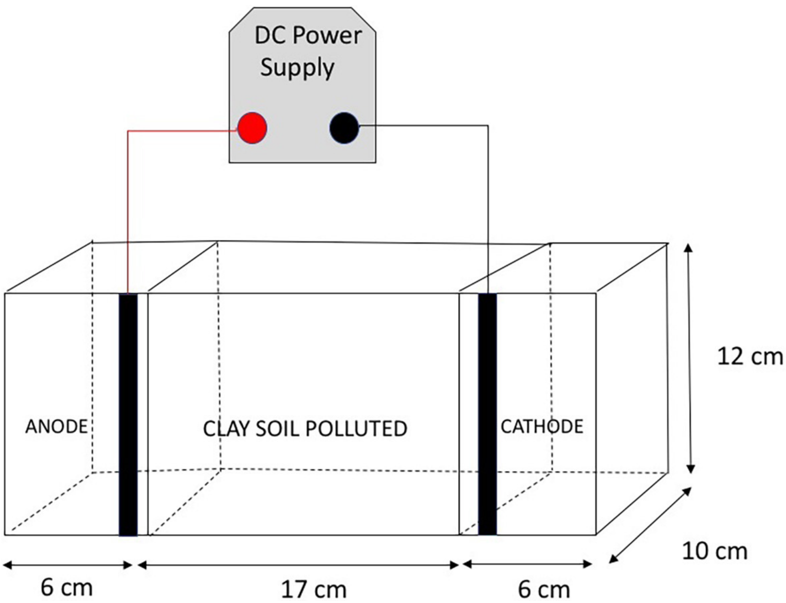


Figure 2. (a) The electrokinetic remediation reactor, (b) The electrokinetic remediation reactor setup

from three vertical layers (top, middle, and bottom) to evaluate total petroleum hydrocarbon (TPH) removal efficiency. The control reactor received no electric current and no MES surfactant addition. Figure 3 presents the sampling scheme during the remediation process.

All treatments were performed in biological triplicates (n = 3). For each biological replicate, soil samples were collected and analyzed in technical triplicate.

Analytical methods

Total petroleum hydrocarbon (TPH)

TPH content were quantified using a gravimetric method. Approximately 20 g of soil were extracted using Soxhlet extraction with n-hexane as the solvent for 4 hours at 20 rpm. The extract was filtered through Whatman No. 42 filter paper and concentrated using a rotary evaporator at 70 °C. The residual oil was dried in an oven at 105 °C to constant weight and weighed using an analytical balance. The efficiency of TPH removal was determined according to Equation (1) (Rocha et al., 2019; S. Wang et al., 2025) :

$$\textit{Removal Efficiency}\ (\%) = \frac{C0-Ct}{C0} \times 100\% \quad (1)$$

where: C_0 – the initial TPH content (mg/Kg) and Ct – the residual concentration after treatment.

Figure 4 presents the extraction process.

Microbial population growth

Microbial activity was evaluated by quantifying the total viable petrophilic bacterial population using the total plate count (TPC) method. This involved preparing serial dilutions of each sample, plating them onto nutrient agar (NA), and incubating at room temperature for 48 hours. Results are expressed as colony-forming units (CFU) (Ajona and Vasanthi, 2021; Ma et al., 2020). Figure 5 presents the Population of petrophilic bacteria in Nutrient Agar (NA) media.

Anode	A1	B1	C1	Cathode
	A2	B2	C2	
	A3	B3	C3	

Figure 3. Sampling scheme during the remediation process

The Microbial fold increase was calculated according to Equation 2.

$$\textit{Microbial increase}\ (\textit{Fold}) = \frac{Ct}{Co} \quad (2)$$

where: C_0 – the initial microbial population (CFU/g) and Ct – microbial population (CFU/g) after remediation.

Statistical analysis

This study used an experimental method with a 3 × 3 factorial randomized block design. There were two independent variables: voltage at 17V (1 V/cm), 34 V (2 V/cm), and 42.5V (2.5 V/cm), and remediation time at initial remediation (Initial), after electrokinetic remediation (EKR), and after bioelectrokinetic remediation (BIO-EKR) process levels. The dependent variables were TPH concentration and microbial population. Multivariate analysis (MANOVA) of varying voltage levels on the bioavailability of weathered petroleum hydrocarbons during remediation process was determined by total petroleum hydrocarbon (TPH) concentration removal and bacterial population growth data. It was conducted using statistical software SPSS 16. Data are presented as mean ± standard deviation (SD) of triplicate measurements (n=3). Statistical significance was determined at the 95% level ($p \leq 0.05$).

RESULTS AND DISCUSSION

Effect of voltage gradient on total petroleum hydrocarbon removal

The voltage gradient applied in the experiment strongly affected the availability of weathered petroleum hydrocarbons, as demonstrated by the total petroleum hydrocarbon (TPH) removal efficiency, measured during 48 hours electrokinetic remediation (EKR). After EKR, a bioremediation was carried out and monitored for 33 days. The average removal efficiency of petroleum hydrocarbons during remediation for each treatment is shown in Table 1.

Table 1 shows that Reactor B with 34 V(2 V/cm) achieved the highest removal efficiency (achieving 30.97% TPH removal after 48 hour of EKR and increasing to 85.75% following 33 days

Table 1. The removal efficiency of TPH during remediation

Treatment	Electrokinetic remediation (48 hours)			Bioremediation (33 days)			BIO-EKR Efficiency removal (%)
	Initial TPH (mg/Kg)	Final TPH (mg/Kg)	Efficiency removal (%)	Initial TPH (mg/Kg)	Final TPH (mg/Kg)	Efficiency removal (%)	
A	26,800	22,600	15.67	22,600	5,259.13	76.73	80.38
B	26,800	18,500	30.97	18,500	2,635.97	85.75	90.16
C	26,800	24,500	8.58	24,500	10,100.00	58.78	62.31

Note: A = 17V (1 volt/cm), B = 34 V (2 volt/cm), C = 42.5V (2.5 volt/cm).

(a)

Figure 4. Extraction process: (a) soxhlet extraction, (b) rotary evaporator, (c) extracted oil

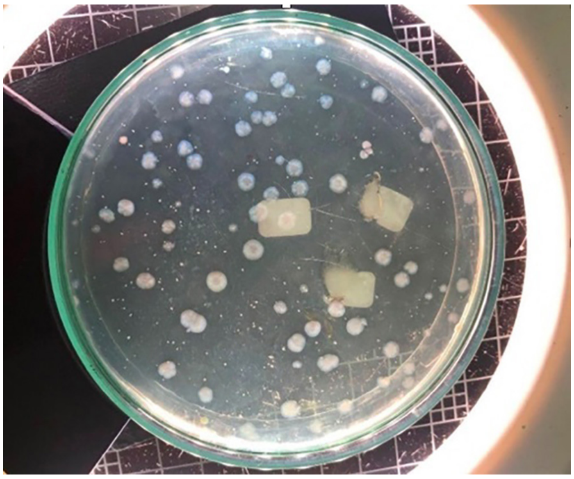


Figure 5. Population of degrading bacteria in Nutrient Agar (NA)

of bioremediation or TPH removal efficiency is 90.16% during 35 days BIO-EKR). The moderate voltage showing improves both remediation. Meanwhile, Reactor A 17 V (1 V/cm) at the end of the 35-day remediation process had a removal efficiency of 80.38% and Reactor C 42.5 V (2.5 V/cm) had the lowest efficiency of 62.31%. the superior results in Reactor B suggest this voltage produces more current, increasing pollutant mobility and the proliferation of degrading bacteria. In contrast, lower efficiency in Reactor C may result from excessive polarization, which reduces ion mobility and inhibits microbial activity, both of which are crucial for remediation. This result is in line with (F. Li et al., 2020), reported that a voltage gradient of 2 V/cm was identified as the optimal electric field strength in increasing the degradation rate of PAHs. Thus, voltage choice directly influences removal efficiency and mechanisms in clay-rich soil.

During the 48-hour EKR process, the current intensity varied depending on the applied voltage: 17 V (1 V/cm), 34 V (2 V/cm), or 42.5 V (2.5 V/cm). Based on these findings, Reactor B at 34V (2 V/cm) maintained a steady current of about 0.25A after 40 hours electrokinetic remediation. This indicated stable electrochemical conditions and efficient ion migration (Figure 6). The 34V (2 V/cm) setting was most effective for clay remediation of weathered petroleum hydrocarbons contaminated soil. It provided an optimal current density that enhanced pollutant transport and microbial degradation. These conditions in Reactor B achieved electrokinetic remediation (EKR) within 48 hours. This is much shorter than the 240 hours reported by Gidudu and Chirwa (B Gidudu and Chirwa, 2020a). Previous studies most often used electrical current for 7–20 days in electrokinetic soil remediation to remediate petroleum hydrocarbons while some wastewater pretreatment or electrochemical studies used only a few minutes to 24–48 hours (Saini et al., 2020; Crognale et al., 2020). These results show in addition to the chemical advantages of MES, this study also significantly lower energy consumption and remediation time.

Figure 6 shows the current intensity variation during a 48-hour EKR. Reactor A peaked at 0.10 A at 40 hours, then decreased to 0.08 A by 48 hours. Reactor B reached 0.26 A at 15 hours and stayed near 0.25 A by experiment's end. Reactor C attained 0.24 A at 48 hours. These trends reflect electrochemical reactions and ion migration in soil. Electric current flow induces electroosmosis, electrophoresis, migration, and redox reactions, all aiding mass transport (Fan et al., 2020). The initial current increase is due to water electrolysis, generating H^+ (anode) and OH^- (cathode), which boosts conductivity. Later decline is due to electrode polarization and greater resistance at the electrode–electrolyte interface (B Gidudu and Chirwa, 2020b). During the EKR process, H+ and OH- are constantly produced due to the stable voltage, which decreases the electrical gradient. This also causes H+ and OH- to move into the soil, affecting the direction of electroosmotic flow (EOF). When the cathode reaction is intermittent, this creates a depolarization effect that helps maintain the electrical current and soil pH, because fewer OH- ions are produced during these breaks (Jeon et al., 2015).

The changes in soil pH during the BIO-EKR process are presented in Table 2. Overall, a slight decrease in pH was observed within the first 48 hours to 7 days of treatment across all voltage treatments compared to the initial pH. Subsequently, the pH tended to increase towards the end of the remediation period. The control reactor without voltage showed relatively stable pH values throughout the experiment. The observed pH fluctuations are likely due to electrochemical reactions at the electrodes and microbial metabolic activities during hydrocarbon degradation.

The variation in soil pH is an important factor in BIO-EKR systems. As shown in Table 2., the initial decrease in pH, especially in Reactor B and C with higher voltage, can be attributed to the generation of H+ ions at the anode due to water

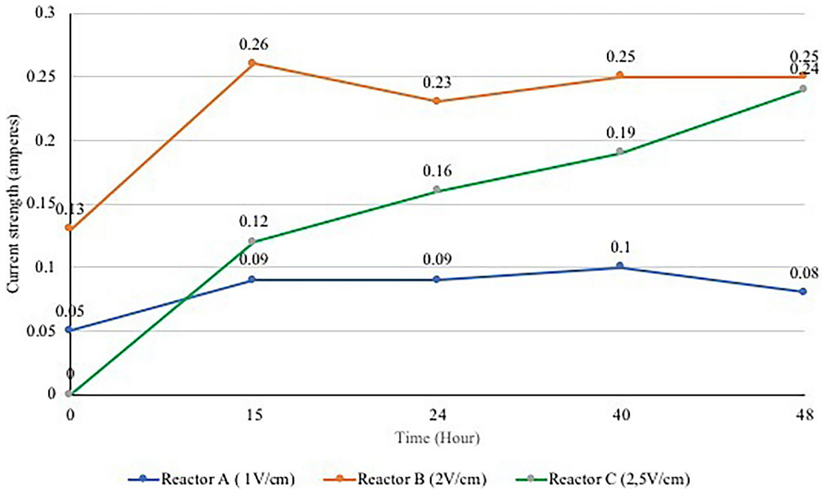


Figure 6. Current strength during 48 hour electrokinetic remediation (EKR)

Table 2. Changes in soil pH during BIO-EKR treatment

Treatment	0 Hour	24 Hour	48 Hour	7 Day	14 Day	21 Day	35 Day
A	8.07	7.95	8.04	7.82	8.19	8.44	8.22
B	8.08	8.03	7.90	7.60	7.77	8.03	8.03
C	8.52	7.98	9.27	7.90	8.12	8.25	8.10
Control	8.31	8.38	8.77	7.76	8.04	8.20	8.22

Note: A = 17V (1 volt/cm), B = 34 V (2 volt/cm), C = 42.5V (2.5 volt/cm), n = 3.

electrolysis (Jeon et al., 2015). The subsequent increase in pH may be related to the production of OH- at the cathode and buffering capacity of the soil. These pH changes may have influenced TPH bioavailability. Mildly acidic to neutral conditions (pH 7.5–8.5) are generally favorable for hydrocarbon-degrading bacteria (Cookson, 1995; Eweis et al., 1998). The maintenance of microbial activity at 28 g/L MES is further supported by the pH data presented in Table 2, which remained largely within the optimal range for hydrocarbon-degrading bacteria throughout the treatment period. The fact that pH remained within this range for most of the treatment period suggests that the microbial activity was not significantly inhibited. Therefore, the enhanced TPH removal observed in this study may be partially attributed to the optimal pH conditions created by the BIO-EKR process.

To determine the effect of variations in electrical voltage levels on the bioavailability of weathered petroleum hydrocarbons, a multivariate analysis of variance (MANOVA) was conducted with statistical significance at the 95% level ($p \leq 0.05$), with respect to the reduction in total petroleum hydrocarbon (TPH) concentration and bacterial population growth. The effectiveness of remediation process at different voltages on TPH removal and microbial population is presented in Table 3, and Summary of the MANOVA and between-subjects effects test results is presented in Table 4.

The results shown in Table 3, the initial TPH concentration in the contaminated soil was 26,800.00 mg/kg with a microbial population of 3.00×10^6 CFU/g. The application of electrokinetics remediation (EKR) technique before the bioremediation process significantly increased the TPH removal at the end of the remediation process (bioelectrokinetic remediation (BIO-EKR)). The highest TPH removal efficiency of 90.16% was achieved by the BIO-EKR treatment at 34.0 V (2 volt/cm), with a final TPH concentration of 2,635.97 mg/kg. The lowest removal efficiency at the end of the BIO-EKR process of 63.31% was observed at 42.5 V (2.5 volt/cm), indicating that excessive voltage can inhibit microbial activity and reduce remediation efficiency.

The results of the MANOVA test based on Table 4 show that remediation time has a very significant effect on the TPH concentration $F(2, 18) = 90.000$, $p < 0.001$ and the microbial population $F(2, 18) = 9.221$, $p = 0.002$. Voltage significantly affects the TPH concentration $F(2, 18) = 3.808$, p

Table 3. Effectiveness of bio-electrokinetic remediation on TPH removal and microbial population

Treatment	TPH (mg/Kg)		TPH removal efficiency (%)	Microbial population (CFU/g)		Microbial increase (fold)
	Initial	Final		Initial	Final	
A	26,800.00	5,259.13	80.38	3.00 ×10^6	18 .00 ×10^7	60
B	26,800.00	2,635.97	90.16	3.00 ×10^6	16.00 ×10^7	53
C	26,800.00	10,100.00	62.31	4.00×10^6	3.00 ×10^8	75

Note: A = 17V (1 volt/cm), B = 34 V (2 volt/cm), C = 42.5V (2.5 volt/cm).

Table 4. Summary of MANOVA and between-subjects effects test of voltage and remediation time on TPH concentration and microbial population

Source	Variable dependent	df	F	p-value	Sig.
Remediation time	TPH concentration	2	90.000	<0.001	*
	Microbial population	2	9.221	0.002	*
Voltage	TPH concentration	2	3.808	0.042	*
	Microbial population	2	0.690	0.520	ns
Voltage × Time	TPH concentration	4	0.850	0.512	ns
	Microbial population	4	1.120	0.378	ns

Note: * – significant ($p < 0.05$), ns – not significant

= 0.042 but does not affect the microbial population $p = 0.520$. There is no significant interaction between voltage and remediation time for both parameters $p > 0.05$.

The statistical analysis revealed that remediation time was the most significant factor affecting TPH removal and microbial population growth, in other words, remediation time was the most dominant factor affecting BIO-EKR remediation performance. The BIO-EKR process applied for 35 days preceded by a 48-hour electrokinetic remediation process, followed by a 33-day bioremediation process, had an impact on TPH bioavailability. Although voltage played an important role in TPH removal, its effect on microbial growth at the end of the remediation process was negligible. The absence of interaction indicates that the effects of voltage and remediation time on TPH degradation and microbial population were independent.

In contrast, the electric voltage showed a significant effect on TPH concentration ($p = 0.042$), and no significant effect on microbial population ($p = 0.520$). The lack of significant effect of voltage on microbial growth at the end of the remediation process (35 days BIO-EKR) can be attributed to the role of the electric field which can increase nutrient distribution and stimulate microbial activity. At a voltage of 34.0 V (Reactor B), the electric field increased nutrient distribution and stimulated microbial activity, resulting in effective TPH removal, and at the end of the remediation process (35 days) the microbes had entered the death phase due to nutrient limitations. However, at a higher voltage of 42.5 V (Reactor C) it resulted in the highest population increase of 75 times because of TPH in reactor C was still sufficiently available for microbial growth but the high voltage in reactor C could also cause stress conditions such as pH gradients that inhibit hydrocarbon-degrading enzymes or in other words it could inhibit microbial activity in degrading weathered petroleum hydrocarbons.

The absence of a significant interaction between voltage and remediation time ($p > 0.05$) indicates that the effects of these two parameters are independent. Therefore, BIO-EKR optimization can be performed separately: 34.0 V is recommended for maximum TPH removal, while 42.5 V can be applied when the goal is microbial bioaugmentation. These findings highlight that selecting the appropriate voltage and treatment duration is crucial to balance contaminant removal efficiency and microbial sustainability in BIO-EKR applications.

Electroosmotic flow and electrolyte transport

Electrolyte volume changes during the 48-hour EKR process indicate electroosmotic flow from anode to cathode. Higher voltage increases migration speed, confirming that the electric potential gradient drives fluid transport in clay soil (Figure 7).

Figure 7 shows that higher voltages accelerate electrolyte transport, emphasizing electroosmotic flow. This observation aligns with (Fdez-Sanromán et al., 2021), who identified electromigration, electroosmosis, and electrophoresis as key mechanisms for contaminant movement. According to Cameselle (2015), electro-osmotic flow occurs due to electrochemical interactions between electric fields, soil particles, and the fluids present between them. Notably, electroosmotic flow aids pollutant transport in low-permeability soils, with its efficiency depending on current, electric field, and soil properties such as pH and moisture (Guo et al., 2014). However, it is important to note that higher voltages may cause pH gradients and polarization, which can decrease performance at 42.5V (2.5 V/cm).

Role of soil moisture and microbial activity

The presence of water in soil is crucial for effective electrokinetic and biological remediation. Sufficient water ensures electrical conductivity, facilitates ion movement, and enables contaminant removal. Optimal soil moisture, typically 20 to 50 percent, supports both conductivity and microbial activity (Cameselle and Gouveia, 2018). Soil moisture also affects the success of bioremediation that occurs after electrokinetic remediation (EKR) process by determining contaminant availability, bacterial activity, and oxygen transport (Cookson, 1995; Eweis et al., 1998). The soil moisture and total bacterial population during remediation are presented in Figure 8.

Based on Figure 8, it can be seen that soil bacteria are very responsive to soil moisture levels, moderate moisture supports the greatest bacterial activity. If the soil is too dry, soil bacteria lack the water needed for metabolism; if too wet, oxygen becomes insufficient, hindering hydrocarbon breakdown. Maintaining optimal moisture directly promotes bacterial growth and increases its activity in degrading total petroleum hydrocarbons (TPH).

Electrokinetic–bioremediation synergy

The remediation efficiency of weathered hydrocarbons in low-permeability clay soils has long been hampered by transport and bioavailability constraints. The results of this study suggest that these limitations can be mitigated by Methyl Ester Sulfonate (MES)-assisted electrokinetic bioremediation. It is hypothesized that MES, as a synthetic anionic surfactant, reduced interfacial tension and promoted micelle formation, thereby facilitating the desorption of sequestered hydrocarbon fractions from soil particles. Furthermore, when coupled with electroosmotic flow induced by the applied electric field, MES-assisted desorption may have enhanced the redistribution of hydrocarbons toward biologically active zones. This combined effect likely alleviated mass transfer limitations inherent to low-permeability soils. The MES concentration used in this study was 28 g/L. At this concentration, microbial activity was maintained despite electrokinetic stresses such as pH gradients and redox fluctuations near the electrodes. This observation suggests that MES primarily functioned as a bioavailability enhancer rather than as a microbial inhibitor, activator

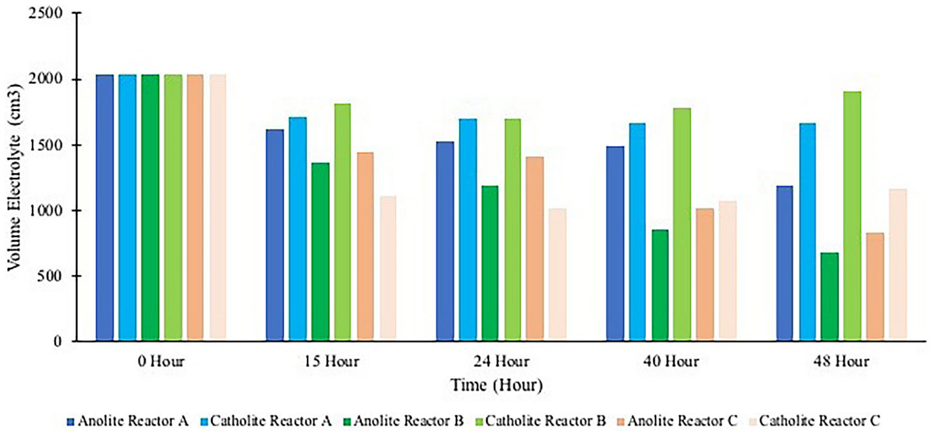
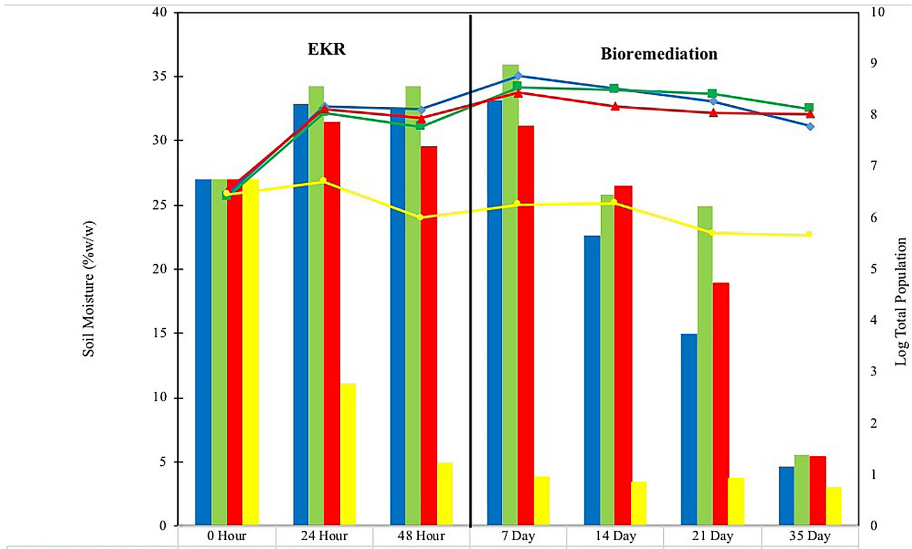


Figure 7. Volume of electrolyte during 48 hour electrokinetic remediation (EKR)



	0 Hour	24 Hour	48 Hour	7 Day	14 Day	21 Day	35 Day
Soil Moisture Reactor A	26.96	32.84	32.45	33.16	22.65	15.01	4.65
Soil Moisture Reactor B	26.96	34.22	34.23	35.92	25.86	24.96	5.49
Soil Moisture Reactor C	26.96	31.4	29.53	31.1	26.47	18.94	5.4
Soil Moisture Control	26.96	11.15	4.96	3.83	3.49	3.71	3.07
Population Reactor A	6.46	8.17	8.11	8.77	8.52	8.27	7.79
Population Reactor B	6.41	8.05	7.78	8.55	8.5	8.41	8.12
Population Reactor C	6.54	8.13	7.94	8.44	8.18	8.05	8.02
Population Control	6.46	6.71	6	6.26	6.28	5.7	5.66

Figure 8. The Soil moisture and Total bacterial population during remediation

agent, or competing carbon source. However, direct measurements of interfacial tension and micelle formation were not conducted in this study. Therefore, the proposed mechanisms are based on the observed remediation performance and are consistent with established literature on surfactant-assisted electrokinetic remediation.

Optimizing the voltage gradient is an effective pretreatment for remediating weathered petroleum hydrocarbons-contaminated soils, as it enhances the interaction between electrokinetic and biological processes. Applying a moderate voltage that sustains stable electroosmotic transport increases microbial access to substrates and facilitates their removal. This method accelerates total petroleum hydrocarbon (TPH) removal and promotes bacterial growth compared to both lower and higher voltage conditions. The 34 V treatment achieved the highest overall TPH removal, indicating optimal coupling between electrokinetic and biological remediation (Figure 9).

The performance of each reactor after electrokinetic remediation (48 hours) is illustrated in the TPH mass balance graph presented in Figure 10.

Figure 10 shows that different voltage gradients resulted in varying remediation efficiencies. As shown, the highest TPH removal during electrokinetic remediation, 32.54%, was observed in Reactor B at 34 V (2 V/cm). Reactor B initially contained 107,177 mg of TPH, and 34,844 mg was removed after 48 hours, corresponding to a removal rate of 725.92 mg/h. Only 0.028% of TPH was detected in the electrolyte, primarily in the anolyte, indicating minimal oil migration from the soil. Therefore, TPH removal was likely attributable to physicochemical degradation resulting from resistive heating as electrical current passed through the soil. These findings are consistent with Fan et al. (2020) who reported that moderate voltage levels enhance ion movement and electroosmotic flow, thereby improving hydrocarbon desorption and degradation.

Following the electrokinetic phase, the impact of the bioremediation stage became clear. After the 33-day bioremediation phase (Figure 11), Reactor B (34 V, 2 V/cm) again showed the highest performance. TPH removal reached 90.87%, with 97,394 mg degraded at an average rate of 115.94 mg/h. This shows that applying moderate voltage improved bacterial growth and activity, enhanced substrate distribution, and increased the bioavailability of petroleum pollutants due to electrokinetic pretreatment.

During the electrokinetic stage, colloidal petroleum hydrocarbons migrate from the cathode to the anode as a result of electrophoresis. This process distributes TPH throughout the soil matrix, increasing their bioavailability to degrading microorganisms. Consequently, biodegradation efficiency improves during the subsequent

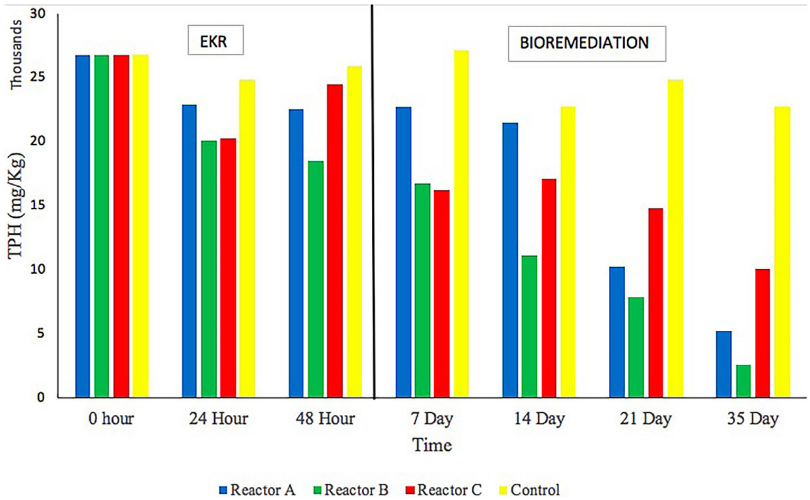
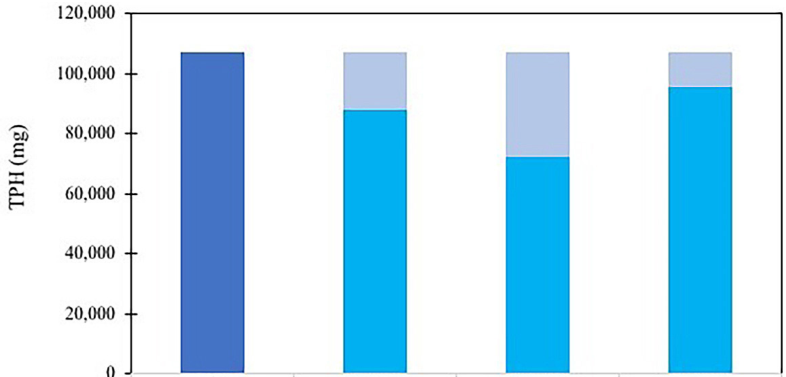


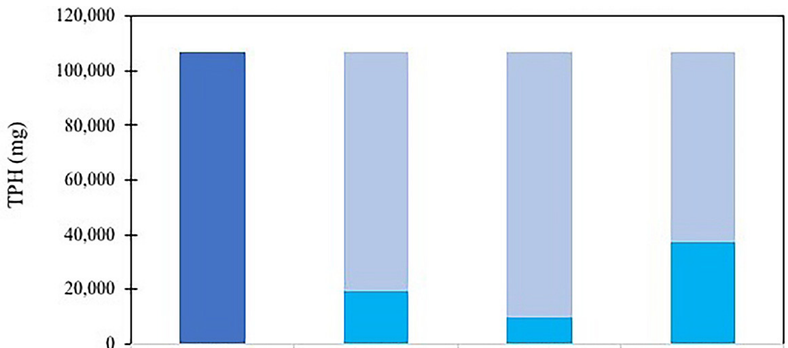
Figure 9. Total petroleum hydrocarbon (TPH) concentration during remediation



	Initial	Reactor A	Reactor B	Reactor C
TPH Removal	0	19,063	34,844	11,445
TPH in Catholite	0	5.9	3.7	5.8
TPH in Anolite	0	83.2	26.6	34.8
TPH in Soil	0	88,025	72,302	95,692
Initial TPH	107,177	0	0	0

Initial TPH · TPH in Soil · TPH in Anolite · TPH in Catholite · TPH Removal

Figure 10. Mass balance of TPH after electrokinetic remediation (EKR)



	Initial	Reactor A	Reactor B	Reactor C
TPH Removal	0	87,629	97,394	69,908
TPH in Catholite	0	5.9	3.7	5.8
TPH in Anolite	0	83.2	26.6	34.8
TPH in Soil	0	19,459	9,753	37,228
Initial TPH	107,177	0	0	0

Initial TPH · TPH in Soil · TPH in Anolite · TPH in Catholite · TPH Removal

Figure 11. TPH mass balance after combined electrokinetic and bioremediation processes (35 days BIO-EKR)

bioattenuation phase. Varjani and Upasani (2017) explained that the availability of petroleum contaminants to petrophilic microbes is a major determinant of the effectiveness of bioremediation processes. Factors such as pollutant volatility, hydrophobicity, and specific soil characteristics influence bioavailability.

Increased electrochemical activity near the anode zone likely promoted localized oxidation of hydrocarbons, thereby facilitating microbial degradation. These findings align with previous research (Brian Gidudu and Chirwa, 2020a; Guo et al., 2014), which highlights the synergistic interaction between electrokinetic processes and microbial activity in enhancing clay soil remediation.

Effect of voltage variation on total bacterial population

Populations of petrophilic bacteria were monitored as primary indicators of remediation performance. Figure 12 presents the logarithmic increase in total bacterial populations within each reactor over the 35-day remediation period.

Figure 12 indicates that the application of an electric field led to a marked increase in total bacterial populations in all reactors relative to the control. For instance, Reactor B, operated at 34 V (2 V/cm), showed an initial population of 3.00 × 10^6 CFU/g, which rose to 16.00 × 10^7 CFU/g after 35 days, corresponding to 53 fold increase compare to the initial population (Table 2). The concurrent increase in bacterial populations and total petroleum hydrocarbon (TPH) removal efficiency suggests that the electrokinetic process enhanced substrate bioavailability and promoted greater microbial activity. Li et al. (2016) found that electric field intensity is important for bacterial growth. This effect likely results from a stress response in the bacteria, and the availability of resources provides the energy for growth. The study also demonstrated that electrolysis at the anode generates dissolved oxygen, which facilitates the growth of aerobic microorganisms.

The highest microbial growth was observed at Reactor C 42.5V (2.5 volt/cm). The difference in performance between 34.0 V and 42.5 V can be explained by two competing mechanisms. At 34.0 V, the electric field was sufficient to enhance desorption and transport of TPH toward the electrodes without causing significant stress to microorganisms. This allowed microbes to actively degrade hydrocarbons, resulting in maximum TPH removal. However, the high metabolic activity led to a decrease in viable cell count at the end of treatment (35 days). At the 35 days the remediation process the microbes in Reactor B 34.0V (2 volt/cm) had entered the death phase due to substrate limitations.

The higher voltage of 42.5 V (2.5 volt/cm), although it promoted microbial transport and resulted in the highest population increase of 75 fold, it may have also induced stress conditions such as pH gradients and Joule heating near electrodes, which inhibited hydrocarbon degradation enzymes. Saini et al. (2020) reported that electrokinetic remediation facilitates microorganism transport in low-permeability soils, where restricted pore size limits microbial movement. Electroosmosis predominantly mobilizes

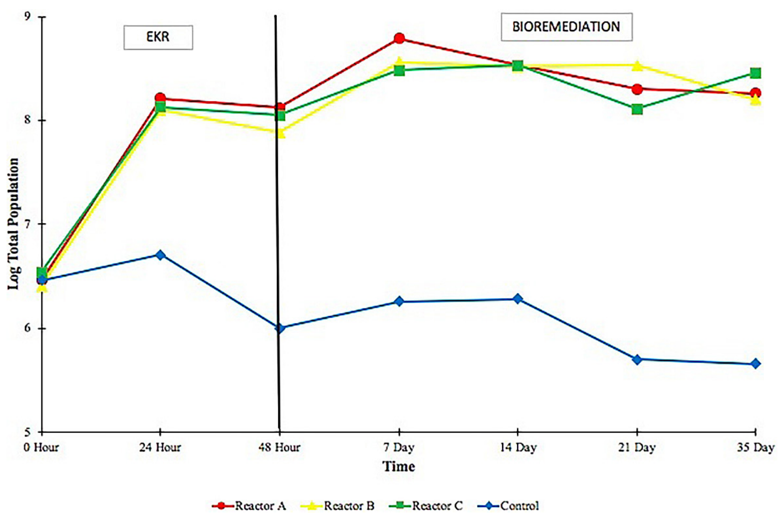


Figure 12. Total bacterial population during remediation

non-motile bacteria, enabling up to 90% movement, compared to 0–20% by electrophoresis. This mechanism is consistent with the present findings, which showed a significant increase in bacterial populations within 48 hours of electrokinetic treatment. During this process, water migrates from the anode to the cathode through electroosmosis, increasing soil moisture content. As a result, the environment becomes more favorable for microbial growth and organic matter degradation (Ossai et al., 2020).

Figure 12 indicates the migration of degrading bacteria by two mechanisms during electrokinetic remediation: electrophoresis moves motile bacteria from the cathode to the anode, and electroosmosis carries nonmotile bacteria from the anode to the cathode. Furthermore, key environmental factors, such as soil pH, humidity, water content, and temperature, significantly influence bacterial population growth (Ossai et al., 2020).

The effectiveness of soil bioremediation is influenced by the abundance of degrading microorganisms, the duration of treatment, the characteristics of specific pollutants and environmental factors (Jasmine and Mukherji, 2015; Mansur et al., 2016). Since physicochemical conditions at contaminated sites largely determine which microbial populations can survive and proliferate by utilizing available contaminants, understanding these factors is essential. Therefore, monitoring the composition of native microbial communities is critical for effective environmental management (Muangchinda et al., 2020).

The results indicate that the electrokinetic process functions as a biostimulation mechanism for indigenous hydrocarbon-degrading bacteria. During the initial 48 hours of electrokinetic treatment, bacterial abundance increased significantly, and this upward trend persisted throughout the 33 days bioremediation phase, aligning with ongoing TPH removal. Furthermore, the bioremediation of weathered hydrocarbons in clay soils was much improved by the electrokinetic application of Methyl Ester Sulfonate (MES), which also improved the bioavailability and transport of contaminants. MES as a surfactant is very suitable for use in this study due to its relatively high biodegradability, low toxicity to microorganisms at moderate concentrations, and stability over a wide pH range. These properties make MES very suitable for increasing the bioavailability of weathered hydrocarbons that is strongly adsorbed on soil particles. Saini et al. (2020) reported that electrokinetic stimulation enhances nutrient and contaminant distribution, thereby increasing microbial access to hydrocarbons. The observed increase in bacterial population in this study supports the conclusion that electrokinetic pretreatment improves both bioavailability and biodegradation potential for remediating weathered petroleum hydrocarbons contaminated clays.

CONCLUSIONS

This study demonstrated that EKR greatly increases the weathered petroleum hydrocarbons bioavailability in clay soil. The applied voltage gradient strongly influenced pollutant migration, electroosmotic flow, and microbial activity. Among the tested voltages, 34 V (2 V/cm) was identified as the optimal condition, achieving 30.97% TPH removal efficiency after 48 h of EKR and up to 85.75 % following 33 days of bioremediation or 90.16% during 35 days of bioelectrokinetic remediation (BIO-EKR). The moderate voltage level promoted stable current density and efficient ion transport, facilitating substrate desorption and microbial degradation. In contrast, excessively high voltage (42.5V) reduced removal performance due to electrode polarization and unfavorable electrochemical reactions.

The bacterial population increased markedly in all treated reactors. This finding indicates that electrokinetic treatment acted as a biostimulation mechanism, facilitating the migration and proliferation of petrophilic bacteria. Moderate voltages supported microbial survival and enzymatic activity, while excessive voltages (>2.5 V/cm) could inhibit bacterial metabolism. BIO-EKR applications at 42.5 V resulted in the highest microbial population of 3.00×10^8 CFU/g, 75 times higher than the initial. Statistical analysis revealed that bioelectrokinetic remediation (BIO-EKR) time had a highly significant effect on both TPH concentration and microbial population. Voltage significantly affected TPH removal but not microbial population. No significant interaction was observed between voltage and time. This study demonstrates that optimizing voltage and time is crucial for effective BIO-EKR application in field-scale remediation.

The findings confirm that electrokinetic pretreatment effectively enhances bioremediation performance by improving weathered hydrocarbon bioavailability and shortening the overall remediation period. The optimal electric field strength 34 V (2 V/cm) enhances contaminant desorption, mass transport, and microbial degradation. The remediation of weathered hydrocarbons in clay-rich soils remains a challenge due to the combined limitations in bioavailability and mass transport, and the synergistic role of MES in enhancing electrokinetically driven bioremediation under these conditions has proven its reliable ability to enhance weathered hydrocarbons bioavailability. This combined technique provides a promising and sustainable alternative for large-scale soil remediation in sites with low permeability and high hydrocarbon contamination.

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