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Performance evaluation of electrokinetic bioremediation for weathered petroleum hydrocarbon-contaminated soil

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Abstract. This study investigates the impact of Electrokinetic Remediation (EKR) time on total petroleum hydrocarbons (TPH) removal from soil, focusing on electroosmotic phenomena guided by Helmholtz-Smoluchowski theory. Soil samples were exposed to a constant 2 V/cm voltage gradient for 8, 16, and 24 hours, utilizing a 0.05 M Na_2SO_4 solution as an electrolyte to enhance soil conductivity. Biostimulation was combined with EKR, and TPH levels and microbial colonies (TPC) were monitored over a 4-week period. Maintaining optimal conditions (25-40°C, pH 6-8, 30-60% water content) was crucial for effective biodegradation. Results showed that a 24-hour EKR duration was most effective, achieving an 87.9% removal rate. The 16-hour duration closely followed at 85.7%, while the 8-hour duration exhibited lower effectiveness at 62.5%. TPC counts increased in the fourth week for the 16-hour and 24-hour durations but decreased for the 8-hour duration. Post-EKR, a gradual decline in TPH levels indicated electroosmotic flow's (EOF) positive impact on TPH desorption and biodegradation. Electroosmosis influence was evident in varying TPH concentrations among segments after EKR, with higher levels near the cathode in the 16-hour and 24-hour durations. Lower TPH near the cathode in the 8-hour variation suggested electroosmosis-induced desorption, while reduced concentrations in regions A (near anode) and B (middle) resulted from biodegradation and microbial mobility due to electrophoresis. Confirmation of electroosmosis across all durations was supported by observed water content and EOF volume. The 24-hour duration exhibited the highest EOF proportion in the cathode chamber at 22.2%, followed by 16 hours at 16.1%, and 8 hours at 8.6%.

1 Introduction

According to data from the Directorate of Contaminated Land Remediation and Hazardous Waste Emergency Responses, Ministry of Environment and Forestry (MoEF) of the Republic

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of Indonesia, the total area of contaminated land in Indonesia is 6,710,628.99 square meters, which is equivalent to 7,987,278.27 tons. Out of this, 6,054,506.22 tons have been managed. The extraction, processing, and utilization of fossil fuels, particularly in oil and gas production, contribute significantly to soil and groundwater pollution in Indonesia. In 2022 alone, the national oil and gas production reached 1.018 million barrels of oil equivalent per day. When oil products are accidentally spilled on the ground surface, they seep into the unsaturated zones and can eventually reach the groundwater, leading to pollution. Numerous studies have explored the application of electrokinetics to enhance bioremediation effectiveness by providing nutrients to indigenous bacteria, thereby promoting their growth and pollutant degradation capabilities [1-3]. This approach holds promise for remediating fields with low hydraulic permeability, which typically pose challenges for traditional bioremediation techniques. In contrast to pressure-driven flow, electrokinetic processes offer advantages such as more uniform flow distribution and improved control over flow direction [4-6]. Moreover, the transport phenomena within electrokinetic remediation, including electroosmosis and electromigration, can effectively remove contaminants from soil solids while increasing the bioavailability of these contaminants for bacterial degradation. Therefore, this contribution aims to assess the effectiveness of electrokinetic remediation treatment in removing TPH from soil, identify the occurrence of electroosmosis and electromigration mechanisms in the EKR process, as well as investigate the occurrence of biodegradation involved in the bioremediation of weathered petroleum hydrocarbon.

2 Methodology

This research involved several stages, which are described as follows:

1. Initial setup: Soil samples were homogenized, and the physical and chemical properties of the soil were characterized. Reactors for pilot-scale electrokinetic bioremediation testing were manufactured. The reactor used in this study is a combination of remediation techniques using Electrokinetic Remediation and Bioremediation via biostimulation. The reactor is made using acrylic glass and divided into three compartments: one containing contaminated soil, with dimensions of 180 mm × 120 mm × 100 mm, which accommodates 1.5 kilograms of soil, and two electrode compartments with dimensions of 55 mm × 120 mm × 100 mm each. The anode is placed in one compartment and the cathode in the other, with an outlet to the electrolyte reservoir (Figure 1).
2. Implementation of electrokinetic bioremediation: The process involved applying 2V/cm electricity with variations in treatment time (8 hours, 16 hours, and 24 hours) using a 0.05 M Na_2SO_4 electrolyte, graphite electrodes, and air pumps on the ground bed that supply aeration from the diaphragm pump at a speed of 2 L/minute to three parts of the reactor (regions A, B, C; at lengths of 6 cm, 12 cm, and 18 cm) through the air stone. After the EKR process, the soil was allowed to sit for four weeks to undergo the biodegradation process.
3. TPH and TPC concentration analysis: The concentrations of total petroleum hydrocarbons (TPH) and total petroleum carbon (TPC) were calculated at the beginning of the EKR process, the end of the EKR process, and during the first to fourth weeks after completion of the EKR process. This analysis aimed to identify the influence of EKR on the bioremediation processes and the electrokinetic mechanisms involved in TPH removal, such as electroosmosis and electrophoresis. Technical n-hexane is used for TPH testing, while NA (Nutrient Agar) is used for Total Plate Count testing. For the TPH extraction method, a four-hour extraction for each sample in a Soxhlet waterbath measuring 96ºC was employed. To conduct total plate count testing, an analytical balance is used to weigh 10 grams of soil samples. The soil sample is mixed with 90 ml of distilled water in an Erlenmeyer flask, shaken to homogenize the solution, and made into a stock

solution. Tenfold serial dilutions of all mixtures are performed by using sterile distilled water as a diluent. Seven test tubes containing 9 ml of distilled water are used for serial dilution. A 1 ml aliquot of the 10^{-3} to 10^{-6} dilution is placed into a duplicated sterile Petri dish and 20 ml of combined liquid nutrient agar with nystatin to suppress fungal growth. The cup is stirred to allow homogenization. The plate is then incubated at 37°C for 24 hours. Finally, the colonies on the petri dish are counted.

4. Analysis of environmental parameters: Soil pH, temperature, and moisture content (oven drying method), as well as current gradient and electrical energy consumption were recorded. Paired with literature analysis, these parameters helped identify the occurrence of electroosmosis, electrophoresis, and electromigration mechanisms during the EKR process.

Fig. 1. EKR-bioremediation reactor set-up

3 Results and discussion

3.1 The impact of electrokinetic treatment time variation on total petroleum hydrocarbon (TPH) concentration reduction

The highest TPH removal effectiveness was observed in the 24-hour treatment duration, followed by the 16-hour and 8-hour durations, with the control group exhibiting the lowest effectiveness. In the 24-hour electrokinetic treatment, the initial TPH concentration of 1.705% was reduced to a final concentration of 0.206%. This indicates a direct correlation between the duration of electrokinetic remediation and TPH removal effectiveness, as illustrated in Table 1. Fluctuations in TPH concentrations were attributed to the transport of contaminants at specific points in the tested soil caused by electroosmosis flow [7]. However, a decreasing trend was observed in all four electrokinetic tests, as depicted in Table 1 and Figure 2. After the electrokinetic process is halted, it is observed that the TPH concentrations in segment C (near the cathode) are notably higher compared to segments A (near the anode) and B (center) for the 16-hour and 24-hour variations. This disparity can be attributed to the occurrence of electroosmosis, which leads to the transportation of hydrocarbon compounds from the positive pole (anode) towards the negative pole (cathode). Electroosmosis stands as the predominant mechanism for transporting inorganic and organic contaminants within solutions or emulsions through soil interstitial fluids [8, 9].

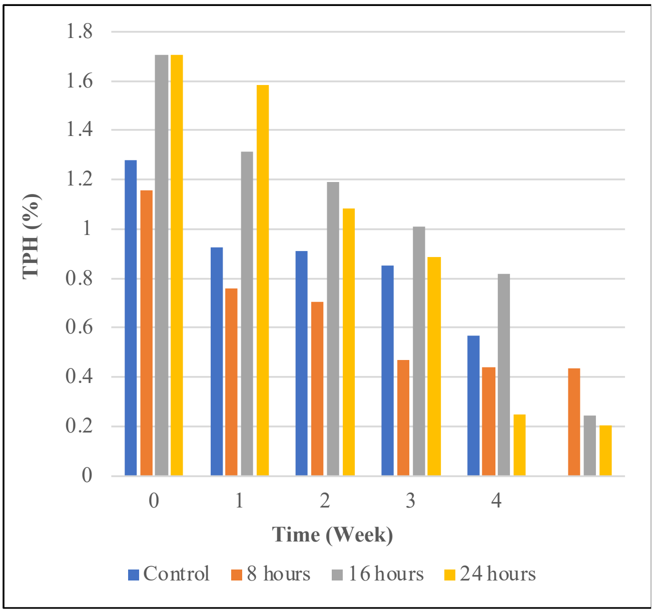

Fig. 2. Average decrease in TPH concentration across different variations of EKR-Bio time

Table 1. Decline in total petroleum hydrocarbon (TPH) concentration with varied electrokinetic treatment durations

Time (Week)	Control	8 hours	16 hours	24 hours
	TPH (%)			
0 (Start of EK)	1.281	1.159	1.708	1.705
t (End of EK)	N/A	0.761	1.315	1.584
1	0.924	0.707	1.193	1.084
2	0.911	0.469	1.012	0.885
3	0.853	0.439	0.820	0.25
4	0.568	0.434	0.245	0.206
Effectiveness	55.7%	62.5%	85.7%	87.9%

Based on Figure 3, the concentration disparity gradually diminishes over time following the deactivation of the power supply (at 8, 16, and 24 hours) until the conclusion of the observation period, which extends until the 28th day. In contrast, an interesting observation is made in the 8-hour treatment variation. Initially, the concentration of TPH was predominantly higher in the region near the cathode (C). However, after 8 hours of electricity application, an unexpected change in distribution occurred. The concentration of TPH in region C (cathode) was lower compared to regions A (near the anode) and B (center). This decrease in TPH concentration of region C can be attributed to the phenomenon of electroosmosis in the electrolyte flow within 8 hours. It causes the desorption of hydrocarbon compounds, leading to their dissolution in the electrolyte solution present in the cathode chamber. Nevertheless, as time progresses, it becomes evident that only region A consistently exhibits a linear decrease in TPH concentration from the first to the fourth week after the power is turned off. This continuous reduction in TPH concentration in region A (and B, albeit with a more pronounced effect in region A, as there is a rebound in region B's TPH level) can be influenced by the combined processes of biodegradation and microbial mobility in the soil, which are associated with the application of electric current [10]. The disparity in TPH content profiles following the cessation of electricity in the 8-hour variation, as compared to the 16 and 24-hour variations, can be attributed to the differing electroosmosis activities. The 8-hour electroosmosis activity only transfers a mass of hydrocarbon compounds from region C to the catholyte solution. Conversely, in the 16 and 24-hour variations, the electroosmosis activity facilitates the transportation of hydrocarbon compounds from regions A and B towards region C and the catholyte solution.

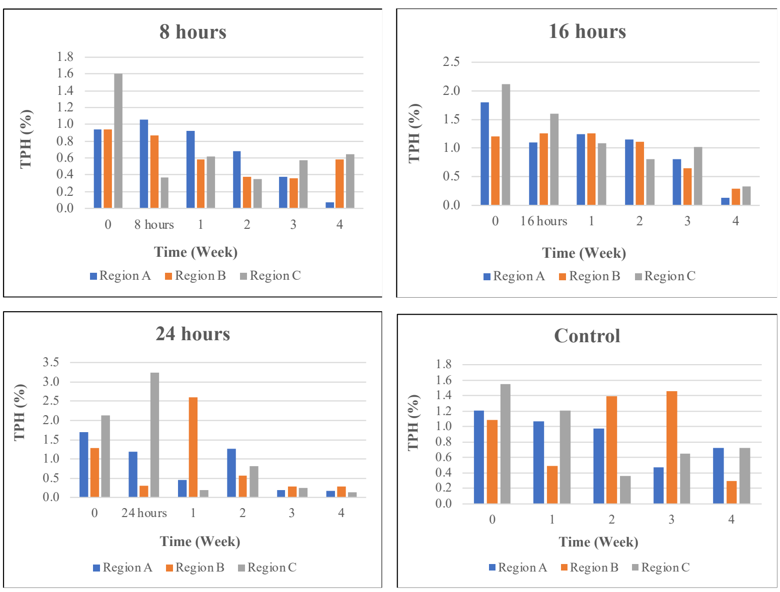

Fig. 3. Distribution of TPH concentrations (%) in segments A (near the anode), B (middle), and C (near the cathode) across all variations

3.2 Influence of time-dependent variations in soil pH, temperature, and water content on transport processes during EKR

3.2.1 pH

During electrokinetic remediation, soil pH near the anode typically ranges from 2 to 3.5, while near the cathode, it ranges between 8 and 11. Optimal pH conditions are crucial for electrokinetic bioremediation to enhance the ability of bacteria to degrade contaminants, as most bacteria thrive in a pH range of 6 to 8 9 [11]. Test results indicate that changes in soil pH in response to electrokinetic behavior vary, tending to stabilize at a near-neutral pH after the power supply is turned off. In the immediate aftermath of power supply cessation, the 16-hour EKR variation displayed a near-acidic pH near the anode (2.05) and an alkaline pH near the cathode (12.20). Conversely, the 8-hour and 24-hour EKR variations maintained pH levels in the near-neutral range (6.41 and 6.02, respectively) (refer to Figure 4). Explaining the disparity in soil response near the anode (region A) between the 8-hour and 16-hour variations, the lower pH can be attributed to the accumulation of acidic byproducts in the vicinity of the anode. As the electrokinetic process advances, water electrolysis occurs at the anode, resulting in the formation of hydrogen ions (H^+) and subsequent acidification of the surrounding soil [3]. Prolonged durations lead to a greater accumulation of these acidic byproducts, resulting in lower pH levels. Interestingly, pH variations in the 8-hour, 16-hour, 24-hour, and control groups did not directly correlate with the growth of indigenous soil microorganism colonies. For instance, the low pH near the anode (region A) in the 16-hour EKR variation after power supply cessation did not impact the increase in microorganism numbers in that region (10.62×10^5 CFU/gr, compared to the previous count of 4.23×10^5 CFU/gr). This indicates the presence of indigenous soil microorganisms that exhibit resilience to low pH conditions.

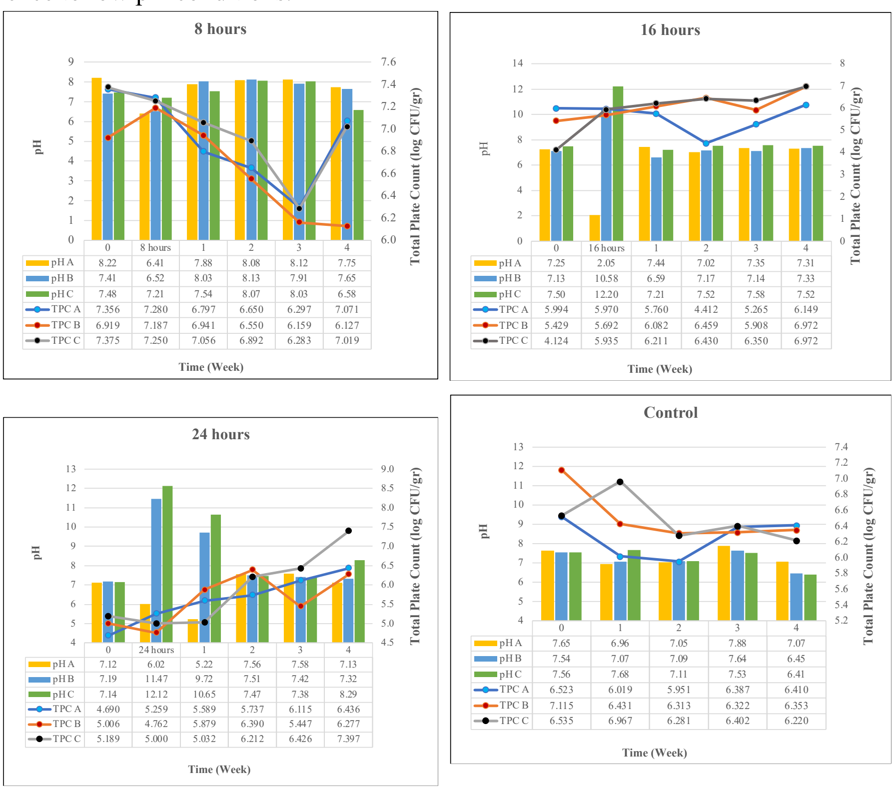

Fig. 4. Comparison of soil pH and microorganism colony count across all EKR variations

3.2.2 Temperature

Microorganism growth rates are generally enhanced by higher temperatures, with optimal degradation ability occurring between 25°C and 40°C [12, 13]. During electrokinetic processes, temperatures typically increase within the range of 5 °C to 20 °C. This temperature rise, induced by the stress gradient applied to the soil, can accelerate the rate of oil degradation by microorganisms, with higher concentrations resulting in a faster temperature increase. The electrolysis reaction of water at the exothermic anode contributes to the temperature elevation, and the generated heat is transferred throughout the soil via the electroosmosis mechanism. Figure 5 demonstrates that the temperature promptly rises to

33°C shortly after the power supply is activated. Throughout the entire screening period, the temperature differences among regions remain within ±0.5 °C. Following power supply cessation, the ground temperature gradually adjusts to the ambient temperature surrounding the reactor, ranging from 24.5 °C to 27 °C over the four weeks after the EKR process. The comparison in Figure 5 reveals the relationship between temperature and the number of indigenous microorganism colonies in the soil across all EKR variations. Notably, the soil temperature range within the reactor consistently remains within the optimal temperature range for the growth of mesophilic bacteria.

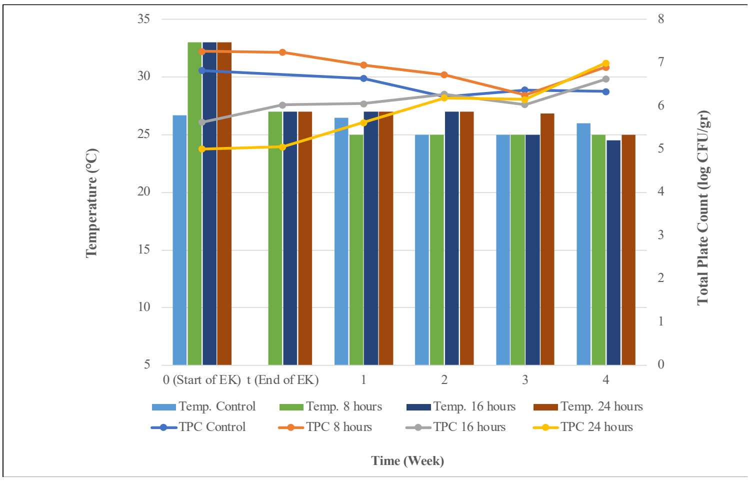

Fig. 5. Graphical comparison of soil temperature and microorganism colony count across all EKR variations

3.2.3 Water content

Water content in polluted soil plays a crucial role in the bioremediation process, as the presence of fluid within soil pores serves as a medium for nutrient exchange and transportation. Furthermore, fluids in soil pores carry dissolved oxygen (DO), which is essential for aerobic bioremediation processes [4]. The moisture profile of the system is depicted in Figure 6 and detailed in Table 2. The increase in water content across the three EKR time variations indicates that the application of electric fields to petroleum-contaminated soil helps maintain water content through electroosmosis (EOF) flow. EOF plays a vital role in contaminant removal, particularly by facilitating the transport of microorganisms and contaminants within the soil. At the conclusion of the electrokinetic process, water content increases in the cathode chamber due to the water electrolysis process at the anode ($2H_2O \rightarrow 4e^- + 4H^+ + O_2$) and cathode ($2H_2O + 2e^- \rightarrow 2OH^- + H_2$). Meanwhile, electroosmosis in the electrolyte chamber leads to a reduction in water volume within the anode chamber [4]. The test results reveal intriguing insights into the relationship between electrokinetic treatment time variations and soil moisture content. The most notable increase in water content occurred in segment C, near the cathode, during the 24-hour variation, rising from an initial 43.2% to 54.4%. Similarly, in the 16-hour variation, the largest increase in water content was observed in region B (the central part), surging from an initial 40.6% to 50.2%.

In contrast, the 8-hour variation displayed a relatively even distribution of soil moisture content across regions A, B, and C, after the completion of the electrokinetic process. These findings shed light on the comparison of electroosmosis phenomena in relation to EKR treatment time. Following the EKR process, maintaining a moisture content within the optimal range of 30-60% becomes crucial for the growth and activity of indigenous microorganisms. However, it is worth noting that in the 16-hour variation, a significant decrease in soil moisture content during the third week led to a decline in colony numbers compared to the previous week (dropping from 18.64 × 10^5 CFU/gr to 10.78 × 10^5 CFU/gr). The Helmholtz-Smoluchowski model suggests that the flow rate in the soil is directly related to the zeta potential, with the range of zeta potential for a kaolinite soil from +0.7 mV at pH of 2 to -54 mV at pH of 10 [14]. Additionally, an increase in pH corresponds to an increase in the electroosmotic permeability coefficient [15].

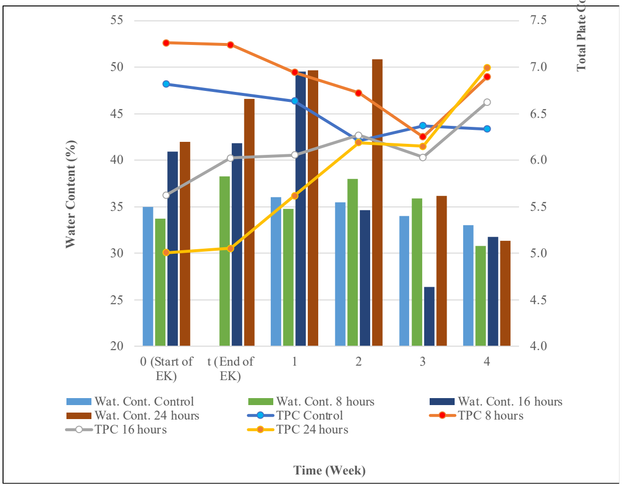

Fig. 6. Graphical representation of comparison graph of soil moisture content and number of colonies of microorganisms across all variations

Table 2. Comparison of pH and moisture content of segments A, B, and C across all variations

Time (Week)	Control						8 hours					
	pH			Water Content (%)			pH			Water Content (%)		
Segment	A	B	C	A	B	C	A	B	C	A	B	C
0 (Start of EK)	7.65	7.54	7.56	35.0	34.7	35.2	8.22	7.41	7.48	33.7	34.8	32.9
t (End of EK)	N/A	N/A	N/A	N/A	N/A	N/A	6.41	6.52	7.21	37.9	38.8	38.0
1	6.96	7.07	7.68	36.2	37.1	34.7	7.88	8.03	7.54	25.7	36.8	41.8
2	7.05	7.09	7.11	35.9	35.5	35.0	8.08	8.13	8.07	38.6	37.6	37.8
3	7.88	7.64	7.53	35.4	32.5	34.0	8.12	7.91	8.03	37.0	34.0	36.7
4	7.07	6.45	6.41	35.2	32.0	31.8	7.75	7.65	6.58	31.0	30.3	31.0
Time (Week)	16 hours						24 hours					
	pH			Water Content (%)			pH			Water Content (%)		

Time (Week)	Control						8 hours					
	pH			Water Content (%)			pH			Water Content (%)		
Segment	A	B	C	A	B	C	A	B	C	A	B	C
0 (Start of EK)	7.25	7.13	7.50	40.5	40.6	41.7	7.12	7.12	7.12	42.1	40.7	43.2
t (End of EK)	2.05	10.58	12.20	39.2	50.2	36.2	6.02	11.47	12.12	37.4	48.0	54.4
1	7.44	6.59	7.21	43.8	49.7	55.1	5.22	9.72	10.65	42.9	48.1	58.0
2	7.02	7.17	7.52	29.9	37.6	36.5	7.56	7.51	7.47	42.1	45.8	64.7
3	7.35	7.14	7.58	23.3	22.1	33.8	7.58	7.42	7.38	31.9	27.9	48.8
4	7.31	7.33	7.52	31.8	31.1	32.5	7.13	7.32	8.29	27.7	30.2	36.0

Table 2 provides a comparison of pH and moisture content in each variation to investigate this phenomenon. Based on the Helmholtz-Smoluchowski theory, the area near the cathode is expected to have the highest zeta potential and the highest water content transported by electroosmosis, demonstrated by a lower water content. However, the results of the moisture test in region C for the 24-hour EKR variation after the power supply is turned off contradict this expectation. Region C displays both the highest pH and the highest water content, suggesting that EKR durations exceeding 24 hours may allow for the complete transportation of electroosmosis flow from the ground to the cathode chamber. It is worth noting that the heating effect can impact the uniformity of electroosmosis flow transfer [16]. Interestingly, the ground temperature shortly after the power supply is turned on appears to be uniform across all three EKR variations. In the 16-hour EKR variation, the highest water content observed in region B (the middle region) indicates the movement of electroosmotic flow from region A (near the anode) to the middle within a 16-hour period. Finally, in the 8-hour variation, the uniform soil water content after turning off the power supply suggests the consistent displacement of electroosmotic flow within the system [17].

3.3 The effect of electrokinetic treatment time variations on volumes of electroosmotic flow (EOF) and electrical power consumption

Electroosmotic flow rate (EOF) represents the movement of water induced by electrical forces, influenced by factors such as soil type, water content, ion type, and ion concentration. Measurements of EOF volume were conducted at the conclusion of EKR treatment, specifically at 8 hours, 16 hours, and 24 hours.

Table 3. Volume of electroosmosis flow of each EKR variation

Day	Parameters	8 hours	16 hours	24 hours
		Electroosmotic flow (ml)		
0	Initial anolyte volume	462	462	462
	Initial anolyte volume	230.34	230.34	230.34
t	Final anolye volume	224.4	6.6	13.2

Day	Parameters	8 hours	16 hours	24 hours
		Electroosmotic flow (ml)		
	Final catholyte volume	250.8	303.6	330
Total volume of EOF		237.6	455.4	448.8
Total volume of EOF in the ground		217.14	382.14	349.14
Percentage of EOF volume in the soil		91.4	83.9	77.8
Total volume of EOF in the cathode chamber		20.46	73.26	99.7
Percentage of EOF volume in cathode chamber		8.6	16.1	22.2

Table 3 provides insights into the differences in EOF volume among the variations. Interestingly, the 16-hour and 24-hour variations exhibit minimal disparity in EOF volume. However, the percentage of EOF volume calculated in the cathode chamber demonstrates a direct correlation with the duration of EKR treatment, with the 24-hour variation showing the highest percentage compared to the 16-hour and 8-hour variations. This finding reinforces the connection between electroosmotic flow and the observed reduction in total petroleum hydrocarbon (TPH) concentration across different regions. Table 4 outlines the estimated electrical energy consumption for each EKR variation, revealing a direct relationship with the duration of EKR variations. Meanwhile, the volume of EOF correlates directly with the electric current gradient [18]. Consequently, the 16-hour EKR variation, which possesses the highest electric current gradient, exhibits the greatest EOF volume. A higher electrical current at the outset of electrokinetic remediation signifies a correspondingly elevated peak current, typically occurring 4 to 5 hours after the commencement of electrical flow [4].

Table 4. Comparison of electrical current and energy consumption across different EKR variations

Parameters	8 hours		16 hours		24 hours	
	t=0 (Initial EK)	t=8 hours (End of EK)	t=0 (Initial EK)	t=8 hours (End of EK)	t=0 (Initial EK)	t=8 hours (End of EK)
Electric Current (A)	0.39	0.5	0.42	0.23	0.39	0.23
Electrical Energy Consumption (kWh)	0	0.032	0	0.109	0	0.138

3.4 Characterization of microorganism profiles following EKR-bioremediation process for identification of biodegradation

The application of electrokinetic bioremediation with varied time durations is anticipated to elicit responses regarding both the removal of total petroleum hydrocarbons (TPH) and the viability of microorganisms involved in the remediation process. Microorganisms can adhere to the surfaces of soil particles or be transported along the electroosmotic flow within the soil pore system [19].

Table 5. Quantification of soil indigenous microorganism colonies across all EKR variations

Time (Week)	Control	8 hours	16 hours	24 hours
	Number of microorganisms (10^5 CFU/gr)			
0 (Start of EK)	65.99	182.34	4.23	1.02
t (End of EK)	N/A	174.06	10.62	1.13
1	43.36	87.89	11.36	4.17
2	16.19	52.72	18.64	15.43
3	23.53	17.80	10.78	14.17
4	21.62	78.52	41.92	98.51

The quantification and distribution of microorganism populations across different electrokinetic time variations is presented in Table 5 and Figure 7. In the 8-hour variation, a decline in microbial colony numbers corresponds to a decrease in the effectiveness of TPH concentration removal from the soil during weeks two to four. However, the decrease in TPH concentrations continues in a linear fashion (refer to Table 5 and Figure 8). This can be attributed to the limited EKR duration, which hinders the optimization of the bioremediation process by impeding the delivery of nutrients, acceptors, electron donors, and contaminants [4].

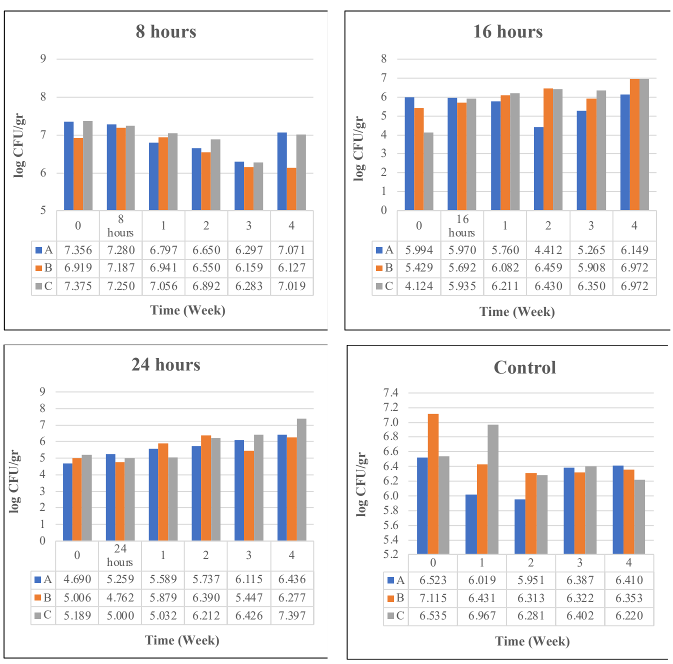

Fig. 7. Distribution of bacterial colonies in different segments across EKR variations

Proliferation does not occur. Instead, a decrease in the number of bacterial colonies is observed in regions B and C, while an increase in colony numbers is observed in region A (as depicted in Figure 7). Overall, the presence of contaminant degradation over the course of four weeks after the power supply is turned off indicates an increased bioavailability of petroleum hydrocarbons (TPH) (see Figure 8). Bioavailability can be defined as the portion of contaminants present in the soil pore fluid at any given time that is accessible for soil biota metabolism [20]. It can also refer to the fraction of contaminants readily consumed by microorganisms [21]. The findings of this experiment align with the assertions made regarding the creation of flow within the electrical double layer through electroosmosis [22]. This flow enhances contaminant desorption and facilitates contaminant degradation activities by bacteria [23, 24].

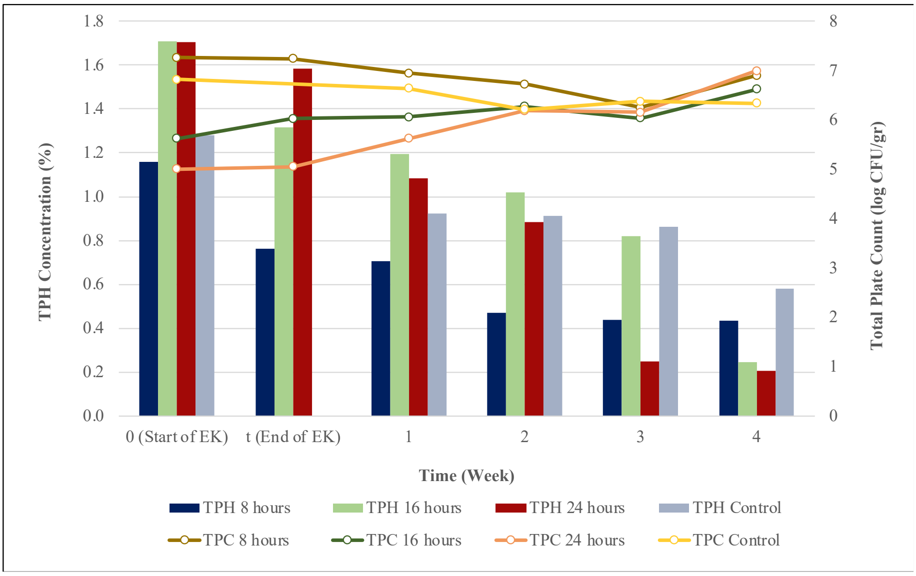

Fig. 8. TPH and TPC profiles across all EKR variations

4 Conclusion

The highest removal effectiveness of Total Petroleum Hydrocarbons (TPH) occurred in the 24-hour running time variation, followed by 16 hours, 8 hours, and the control. In the 24-hour electrokinetic time variation, the initial TPH concentration of 1.705% was successfully removed to a final concentration of 0.206%, indicating that the duration of electrokinetic remediation is directly proportional to the effectiveness of TPH removal. The degradation of contaminants observed for 4 weeks after the electrical flow was turned off suggests an increased bioavailability of TPH. pH and EKR time also play a role in the electroosmosis phenomenon based on the Helmholtz-Smoluchowski theory. Water content tests imply that running times exceeding 24 hours would provide more time for electroosmotic flow to be transported from the soil to the cathode chamber. The 16-hour variation showed the migration of electroosmotic flow from the near-anode segment to the middle segment after the electrical flow was turned off, while the 8-hour variation demonstrated uniform electroosmotic flow (EOF) displacement within the system. The volume of electroosmotic flow (EOF) at the end of the electrokinetic process indicated that the 24-hour variation had the highest percentage of EOF volume, supporting the previous conclusion. The current gradient also had an influence, with the 16-hour EKR variation resulting in the highest volume of EOF. The electroosmotic flow in the 24-hour EKR variation created a flow within the electrical double layer, enhancing the desorption of contaminants and facilitating the degradation activity of bacteria. The 24-hour EKR variation also demonstrated a decrease in TPH concentration in the soil and an increase in microorganism concentration.

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