Pollution Study 2022, 3(1), 2006.
https://doi.org/10.54517/ps.v3i1.2006

Article

Investigation into the electro-microbial approach for the remediation of soil contaminated with oil

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CITATION

Li T, Wu D, Xin L, et al. Investigation into the electro-microbial approach for the remediation of soil contaminated with oil. Pollution Study. 2022; 3(1): 2006.
https://doi.org/10.54517/ps.v3i1.2006

ARTICLE INFO

Received: 9 November 2022
Accepted: 7 December 2022
Available online: 20 December 2022

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Abstract: A graphite electrode was placed at each end of the soil contaminated with oil, and a direct current (DC) voltage of 24 V was applied to decrease the electrical potential across the soil between the electrodes to 1 V/cm. The study examined the impact of the combined electrokinetic and microbial remediation method on various soil parameters such as pH, soil temperature, organic carbon content, and the availability of nitrogen, phosphorus, and potassium in petroleum-contaminated soil. The findings revealed that after the combined treatment, the degradation rate of the simulated soil contaminated with 2% petroleum reached an optimal level of 67.5%. Additionally, the availability of nitrogen, phosphorus, and potassium increased by factors of 1.5, 1.4, and 1.2, respectively. The integration of electric remediation also demonstrated its ability to maintain the soil's pH and temperature within a stable range, thus creating a favorable environment for microbial activity and enhancing the rate of oil degradation.

Keywords: electro-microbial remediation; highly efficient petroleum-degrading bacteria; petroleum

Oil is a typical persistent organic pollutants (pops) in the soil of oil contaminated sites, which contains polycyclic aromatic hydrocarbons (PAHs) that are difficult to biodegrade. Polycyclic aromatic hydrocarbons also produce a large number of toxic intermediates during degradation, polluting soil and groundwater, and eventually entering the food chain [1–4], which may lead to biological mutation.

For decades, bioremediation has been considered as a harmless and ideal remediation method. It does not change the internal properties of soil, nor will it produce secondary pollution in the remediation process. It also has the advantages of relatively low cost, permanent elimination of pollutants and positive acceptance by the public [5–7]. However, its shortcomings of long remediation cycle and low efficiency are important factors that limit the practical engineering application of its technology.

Electrodynamic remediation is an innovative technology to clean up heavy metal or organic contaminated soil, including the control application of low-power DC electric field to contaminated soil. This technology relies on three processes: electromigration, electroosmosis and electrophoresis, and has been used to remove heavy metals and organic pollutants from soil [3,8,9]. Hassan et al. [3] summarized the electrodynamic remediation methods of heavy metals, organic compounds and other dangerous goods, but there are few studies on their use to remove oil contaminated soil such as pentapentapentane, phenol, pentachlorophenol, xylol oil, benzene, toluene and polycyclic aromatic hydrocarbons (PAHs).

Petroleum degrading bacteria can use petroleum as carbon source to maintain their own growth and metabolism. When there is an auxiliary electric field, it can provide continuous heat to enhance the role of highly efficient petroleum degrading bacteria. Traditionally, the slow movement of organic matter and bacteria in the soil is realized by electrodynamic remediation. The elevator degree is used to drive the movement of soil matrix oil, soil nutrients and bacterial cells, and these factors are jointly optimized to improve the efficiency of remediation of oil contaminated soil [10–12]. In this study, a set of electrokinetic microbial combined remediation technology was established to improve the degradation efficiency of organic pollutants in oil contaminated soil, strengthen the effect of bioremediation, and achieve the goal of rapid remediation of organic contaminated soil.

1. Materials and methods

1.1. Experimental materials

1.1.1. Pollutants

The oil collected from the first United Station in South Zone 2 of Daqing Oilfield is taken as the experimental object. The physical and chemical properties of the samples are shown in **Table 1**.

Table 1. Composition and physicochemical properties of crude oil.

Composition and physicochemical properties	Numerical value
P/(20 ℃, g·cm^{-3})	0.882
Alkanes/%	56.12
Aromatics/%	20.11
Resin + asphaltene/%	6.34
Freezing point/℃	25.8
Viscosity/(50 ℃, mPa·s)	18.9

1.1.2. Soil treatment

The original soil samples are from the top soil layer with a depth of 0~20 cm in Shenyang ecological experiment station. See **Table 2** for the basic characteristics of the original soil. In order to evaluate the remediation effect, the artificial simulated oil-bearing soil with uniform oil content was used for the experiment. The oil contaminated soil with oil content of 2% was prepared by mixing the treated soil with a certain proportion of oil. The oil contaminated soil is naturally dried in the soil chamber for 7 days, and then a certain amount is taken in the soil chamber for different treatment (see **Table 3**). The treatment time is 100 days, and samples are taken every 20 days. The sampling point is 0, 4, 8, 12, 16, 20 and 24 cm away from the anode, and the sampling depth is 10 cm. The soil moisture content is about 25%.

Table 2. Basic characterization of original soil.

Project	Conditions and values
Soil type	Brown earth
Mechanical composition	<2 M: 24.8%
pH	2~50 M: 62.6%

Cation exchange capacity/(mol·kg^{-1})	50~2000 M: 12.6%
Organic matter/(g·kg^{-1})	6.5
Total nitrogen/(g·kg^{-1})	0.235
Total phosphorus/(g·kg^{-1})	26.3
Available nitrogen/(mg·kg^{-1})	1.56
Available phosphorus/(mg·kg^{-1})	3.12
Available potassium/(mg·kg^{-1})	92.7
	53.6
	157.1

Table 3. Different treatments of the tested petroleum polluted soils.

		Experimental condition						
Serial number	**Type**	**Anode**	**Cathode**	***U*/(V·cm^{-1})**	**Efficient petroleum degrading bacteria**	**Contaminants**	**Processing time/d**	**Electrode switching**
Experiment 1	Control group	Distilled water	Distilled water	0	-	Petroleum	100	-
Experiment 2		Distilled water	Distilled water	1	-	Petroleum	100	No
Experiment 3		-	-	0	+	Petroleum	100	-
Experiment 4		Distilled water	Distilled water	1	+	Petroleum	100	No
Experiment 5	Test group	NH_4NO_3	KH_2PO_4	1	+	Petroleum	100	No
Experiment 6		Distilled water	Distilled water	1	-	Petroleum	100	Yes
Experiment 7		Distilled water	Distilled water	1	+	Petroleum	100	Yes
Experiment 8		NH_4NO_3	KH_2PO_4	1	+	Petroleum	100	Yes

1.1.3. Composition of microorganism and buffer solution

The mixed culture of petroleum degrading bacteria was used as the experimental bacterial agent, cultured on the shaking table medium at 150 r/min and 30 C, and the culture bacterial agent was harvested by centrifugation at the exponential growth stage, and resuspended in deionized water to obtain 1.2×10^{10} CFU·mL^{-1} high concentration bacterial suspension.

Spray the selected dominant bacteria into the soil, the bacterial content is 10^8 CFU·mL^{-1}, and the dosage is 0.01 mL·g^{-1} soil. Add a certain amount of potassium dihydrogen phosphate solution and ammonium nitrate solution to provide nutrition for bacterial complex, improve soil conductivity and control pH 6.5~7.0. Potassium dihydrogen phosphate (1 mol/L KH_2PO_4) solution is used in the cathode reservoir, and ammonium nitrate (1 mol/L NH_4NO_3) solution is used in the anode reservoir.

1.2. Instruments and equipment

Two groups of electric microbiological laboratory devices were used in this study. The first group of devices is used to optimize the polarity reversal interval of the test. It consists of a soil battery, two electrode units, a pair of cylindrical electrodes made of graphite, an electrode control system, a real-time current and voltage monitoring system and a power supply (see **Figure 1**). The soil unit is made of rectangular plexiglass, with an internal dimension of 26 cm long, 11 cm wide and 10 cm high. Two cylindrical graphite electrodes with a length of 10 cm and a diameter of 0.5 cm are used to generate an uneven electric field. The electrode control device can switch the polarity of the electric field every 24 h.

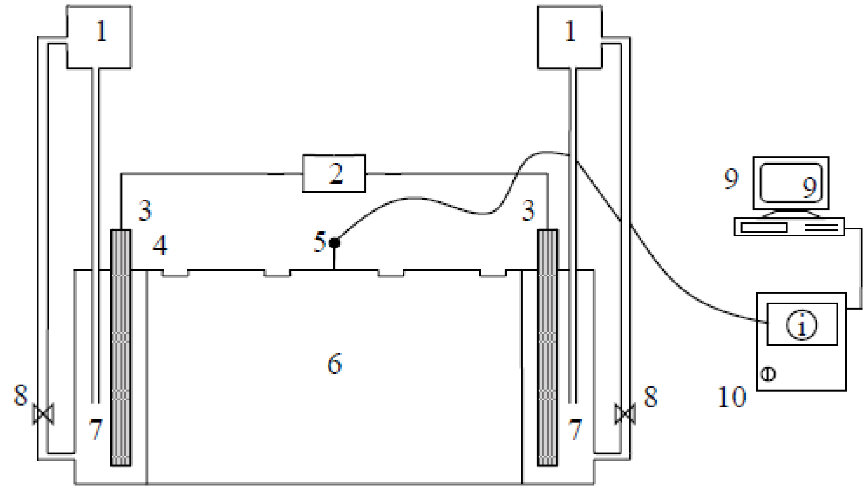

Figure 1. Electric-bioremediation experimental setup.

1 Electrolyte, 2 Power supply, 3 Electrode, 4 Porosity, 5 Thermometer, 6 Soil unit 7 Electrode unit, 8 Osmotic pump, 9 Computer monitoring system, 10 Control system.

Insert an electronic thermometer into the soil to automatically record the temperature and transmit the data to the computer monitoring system. The monitoring system can monitor the current and voltage online, and store the data in the computer for subsequent analysis. The power supply adopts constant DC, and its source drain voltage is 1 v. The potential gradient of the device is 1 v/cm. The second assembly device is consistent with the first group of devices except that the electrode is not converted.

1.3. Analysis method

1.3.1. Soil pH analysis method

The soil pH is measured with a pH meter (phs-3c, shanghai Instrument and electricity company).

1.3.2. Determination of soil available nitrogen, available phosphorus, available potassium and total organic carbon

The available nitrogen of soil is determined by using multi n/c3000 analyzer (Analytik Jena AG, Germany) after alkaline hydrolysis of soil sample; the available phosphorus was extracted by sodium bicarbonate (Olsen method) and determined by molybdenum antimony anti colorimetry; the available potassium was extracted by ammonium acetate and determined by flame spectrophotometry; total organic carbon was measured using a multi n/c3000 analyzer (Analytik Jena AG, Germany). Each index was tested three times [13].

1.3.3. Determination of oil concentration in soil samples

The degradation rate of petroleum in soil was determined by infrared oil detector. Accurately weigh 10 g of soil sample each time, place it in a 50 mL centrifuge tube, add 30 mL of chloroform, cover it, gently shake it for 1 min, and place it overnight. On the next day, put the centrifuge tube in a 55 C water bath for hot soak for 1 h (at the beginning, pay attention to open the cover and let out gas twice), centrifuge, and take the supernatant and put it in a beaker. The soil sample shall be treated twice with the above steps (only the water bath heating time shall be changed to 30 min), and the supernatant shall be taken each time and added to the beaker. Then put the beaker in the fume hood, ventilate and concentrate to dry, which is the chloroform concentrate [14]. Finally, the concentrate was fixed to volume with carbon tetrachloride and its oil content was measured with an infrared oil detector.

2. Results and discussion

2.1. Change of soil pH

Soil pH is generally expressed by pH, which is an important factor affecting the mutual transformation and availability of soil nutrient elements. PH affects the availability of nutrients in soil and the degradation efficiency of pollutants [15].

The initial pH of the soil is 6.5. Taking experiment 2 and experiment 6 as examples, the change results of soil pH at different positions from the anode after 24 hours of experiment are shown in **Figure 2**. In experiment 2, the soil pH changed from 3.5 to 12.7, with a large range. The applied electric field forms an electrolytic cell between the electrodes. The water molecules in the soil undergo electrolytic reaction under the action of the electric field. A large amount of H+ is produced at the anode, which reduces the pH and makes the area around the anode acidic; the cathode produces a large amount of OH−, which increases the pH, and the area around the cathode is alkaline [16]. Strong acidic zone and alkaline zone are formed in the area near the anode and cathode respectively. The extreme environment inhibits the growth of soil microorganisms and is not conducive to the degradation of oil pollutants.

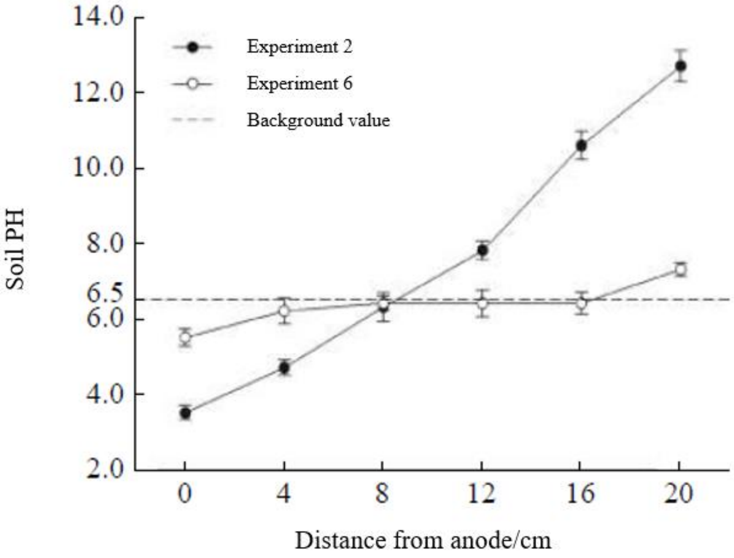

Figure 2. The variation of the tested soils pH affected by electro-microbial remediation treatments.

The soil pH in the area near the anode and cathode of No. 6 soil is 5.5 and 7.3 respectively, and the pH in other locations varies from 6.2 to 6.4, which is slightly lower than the initial soil pH as a whole. This may be because the overall soil pH shows a downward trend due to the electrolysis process [17]. The pH of No. 6 soil is close to neutral, and most of the changes are within the range of microbial fitness (the range of microbial fitness is 6.0~7.5) [8]. It can be seen from the results that through electrode switching, the strong acidic area or strong alkaline area around one end of the electrode is effectively avoided, and the soil pH is controlled within the range suitable for microbial growth. Compared with the soil environment without electrode switching, electrode switching provides a good and stable living environment for microorganisms, which can effectively improve the utilization efficiency of oil by microorganisms.

2.2. Change of soil temperature

Maintaining a constant and appropriate temperature in soil is of great significance to the life activities of microorganisms. Under the condition of 20~45 ℃, the growth rate of microorganisms is fast and their life activities are vigorous [18]. The enhanced activity of microorganisms is conducive to the removal of harmful substances such as oil in the soil [19]. The temperature changes of Experiment 3, experiment 4 and Experiment 7 were monitored for 24 h and recorded every 4 h, as shown in **Figure 3**. The temperature range is 12.4~23.2 ℃, and the maximum temperature difference reaches 10.8 ℃. The range of soil temperature in Experiment 4 and Experiment 7 was not large. In Experiment 4, the maximum soil temperature of 30.3 ℃ appeared at 16 h, the minimum temperature of 29.4 ℃ appeared at 4 h, and the temperature difference within 24 h was 0.9 ℃. In Experiment 7, the maximum temperature of soil was 31.2 ℃ at 12 h, the minimum temperature was 4 h, and the temperature difference within 24 h was 1.8 ℃. In Experiment 3, the temperature changes significantly within 24 h, with a temperature difference of more than 10 ℃, and the soil environment changes relatively greatly. The temperature difference in Experiment 4 is small, with an average temperature of 29.9 ℃, 12.3 ℃ higher than the average temperature in Experiment 3. It is less affected by the external temperature, and the temperature fluctuation is within 1 ℃. The soil environment is relatively stable, providing a good living environment for microorganisms in the soil. In Experiment 7, the temperature rose once in 12 h, and a small temperature fluctuation peak can be seen in **Figure 3**. This may be due to the electrode switching at 12 h, the continuous unidirectional current suddenly changes direction, the surface charge of various charged particles in the soil changes direction, releases energy, and increases the soil temperature. In general, the temperature environment where the microorganism is between 20~45 ℃ and the fluctuation range is small is more suitable for the survival of microorganisms, which will also provide better degradation energy efficiency. The average temperature of Experiment 4 and Experiment 7 is more than 1.7 times that of Experiment 3. The reason may be that part of the electric energy of the applied electric field is converted into heat energy and released into the soil, and the continuous current maintains the soil temperature. The pH of soil is also related to temperature. Constant temperature is conducive to the stability of soil pH. These conditions provide a suitable environment for microbial degradation of oil in soil and increase the degradation efficiency. The degradation rate of Experiment 4 and Experiment 7 was higher than that of Experiment 3, which also verified this view.

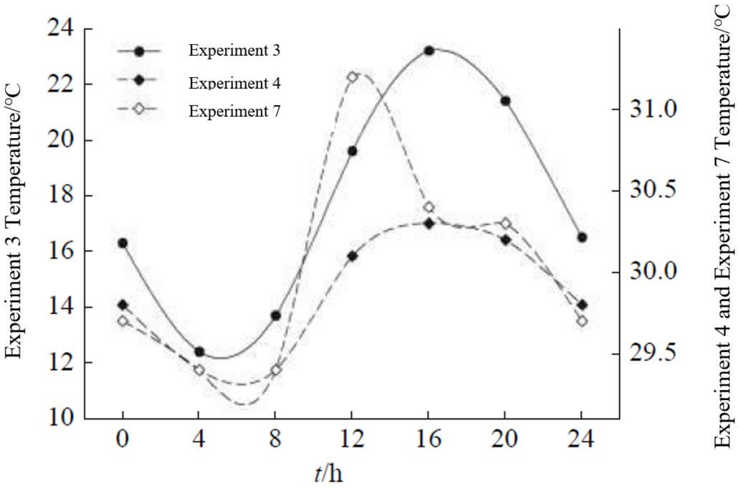

Figure 3. The variation of the tested soils temperature affected by electro-microbial remediation treatments.

2.3. Change of soil organic carbon content

Oil and other macromolecular hydrocarbons can be decomposed into simple organic substances such as small molecule hydrocarbons through the action of highly efficient oil degrading bacteria [20,21]. These organic compounds control the distribution, migration and bioavailability of hydrophobic organic pollutants such as petroleum in the environment. At the same time, the presence of soil organic compounds in soil solution can affect the adsorption of petroleum and other polycyclic aromatic hydrocarbons on soil surface. **Figure 4** shows the change of organic carbon content in Experiment 4 and Experiment 7. As shown in **Figure 4a**, the organic carbon content of each point in Experiment 4 decreased. In the initial 20 days, the consumption rate of anode organic carbon was 9.6% of the total organic carbon, which was 5.65 times faster than that of cathode organic carbon. Experiment 7 shown in **Figure 4b** also has a similar situation. This phenomenon should be attributed to the fact that under the action of electric field, the negatively charged microorganisms migrate to the area with high potential, and a large number of microorganisms gather near the anode; at the same time, the external electric field produces a strong redox reaction at the anode, which stimulates microorganisms to accelerate metabolism and increase the consumption of carbon sources. Organic carbon mostly exists in the form of colloid in soil, with a large number of negative charges. Under the action of electric field, it migrates to the anode by electrophoresis. This also provides a carbon source for microorganisms in the anode region. At the same time, during the migration process, organic carbon adsorbs a large amount of water and cations, and its water absorption is several times or even dozens of times larger than that of clay particles. It can reduce the viscosity of cohesive soil, change the soil pore status and the ratio of water and air, and is conducive to the respiration of soil microorganisms and the transmission of nutrients in the soil.

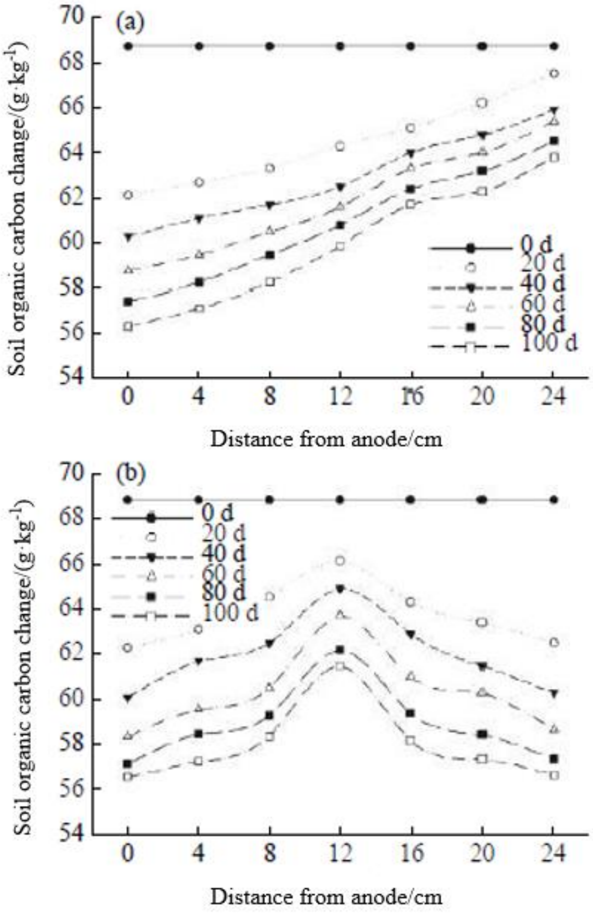

Figure 4. Variation of soil organic carbon affected by electro-microbial remediation treatment.

It can also be seen from **Figure 4** that the rate of organic carbon consumption in the repair process of the same site is also different. As shown in **Figure 4a**, the average consumption of organic carbon in the first 20 days was 6.2% of the total amount. With the extension of repair time, the consumption rate gradually decreased. After 60 days, the average consumption of organic carbon decreased to only 1.6% of the total amount. As shown in **Figure 4b**, the organic carbon content of each point in Experiment 7 is less at the two poles and more in the middle. Switching the electrode makes the two ends of the electrode in the anode state in turn. The oxidation-reduction reaction near the anode is strong, the electron exchange frequency is fast, the life activity of microorganisms is enhanced, the metabolism is accelerated, and the consumption of organic carbon is fast, which can accelerate the degradation of oil by high-efficiency degrading bacteria. The maximum consumption of organic carbon within 20 days reached 9.3% of the total, and the average consumption of organic carbon was equivalent to 7.2% of the total. With the extension of repair time, the consumption rate of organic carbon was first fast and then slow. After 60 days of repair, the consumption rate of organic carbon decreased. In the future experiments, we can consider adding some exotic nutrients as carbon sources.

2.4. Changes of available nitrogen, phosphorus and potassium in soil

Available nitrogen, available phosphorus and available potassium in soil are nutrient elements that can be directly utilized by microorganisms [22]. Taking the changes of available nitrogen, available phosphorus and available potassium in Experiment 4 and Experiment 7 as examples, the effects of electrokinetic microbial remediation on these three nutrient elements were studied. The changes of available nitrogen, available phosphorus and available potassium in soil before and after remediation are shown in **Figure 5**. As shown in **Figure 5**, the contents of available nitrogen, available phosphorus and available potassium in the oil treated soil are not significantly different from those in the clean soil. After 100 days of electrokinetic microbial repair, the available nitrogen, available phosphorus and available potassium in the two groups were significantly higher than those in the control group ($p < 0.05$). In Experiment 4, the available nitrogen and available phosphorus in the anode area are higher than those in the cathode area, which may be because most of the available nitrogen and available phosphorus in the soil exist in the form of negatively charged, and migrate to the anode with higher potential in the electric field [23]. Available potassium exists in the form of K^+ and migrates to the cathode under the action of electric field, so the available potassium in the cathode region is higher than that in the anode region. In Experiment 7, the contents of available nitrogen, available phosphorus and available potassium in the near electrode area were higher than those in the far electrode area, with an average content of 141.52, 49.86 and 187.24 $mg \cdot kg^{-1}$, which were 1.5, 1.4 and 1.2 times that of the control group. The above results show that electrokinetic remediation is beneficial to activate nitrogen, phosphorus and potassium in soil. The increase of available nitrogen may be due to the conversion of macromolecular organic nitrogen into simple organic nitrogen or inorganic nitrogen under the dual action of electric field and microorganism, thus increasing the content of available nitrogen. The available phosphorus in soil mainly comes from inorganic phosphorus, and the phosphorus salt in soil is generally considered to be the most direct source of available phosphorus in soil. In the process of electrokinetic remediation, the pH of the soil decreases, forming acidic conditions, which is conducive to the dissolution of phosphorus salts and insoluble potassium minerals. In addition, the electrolysis process increases the total electrolyte concentration in the soil, which is also conducive to the separation of phosphorus and potassium from the solid phase. Electrolysis produces a large amount of H^+ in the soil system, in which the size of hydrated hydrogen ion (H_3O^+) is similar to that of K^+, and has the opportunity to enter the soil mineral crystal layer to replace K^+, which increases the content of available potassium in the soil. The increase of available nitrogen, available phosphorus and available potassium can provide more nutrition for microorganisms in soil and improve their activity. The content of available nitrogen, phosphorus and potassium is high near the electrode, which may be due to the strong effect of electric field near the electrode.

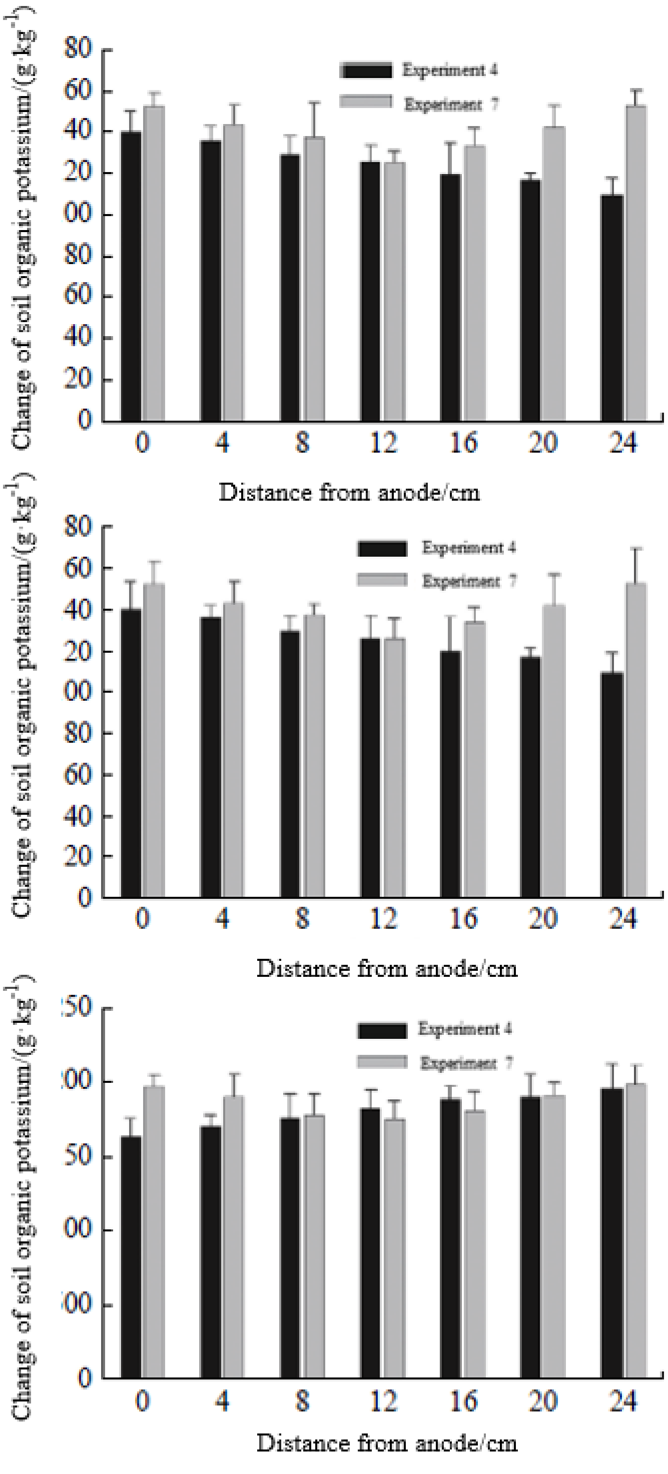

Figure 5. Variation of available nitrogen, phosphorus and potassium affected by electro-microbial remediation treatments.

2.5. Change of oil degradation rate

See **Figure 6** for the change of oil degradation rate under different conditions. As shown in **Figure 6**, single application of electric repair or microbial repair can accelerate the degradation rate of oil. During the repair period, the maximum oil degradation rate in Experiment 2 reached 42.1%, 1.8 times higher than that in Experiment 3, indicating that the electric repair technology is better than the microbial repair technology. In Experiment 4 of the electrokinetic microbial remediation technology, the degradation rate of oil in oil contaminated soil was significantly higher than that in soil with a single applied electric field or microorganism. In Experiment 5, on the basis of electrokinetic microbial remediation, nutrient solution beneficial to microbial growth was added, and the oil degradation rate reached 61%. The electrode is inserted into the contaminated soil, and a DC voltage is applied at both ends of the electrode to form a DC electric field between the two poles. Under the action of electric field, the physical structure of oil pollutant macromolecules may change, making the macromolecules without polarity change into polar charged molecules, and migrate with soil water; the structure of macromolecular pollutants may also be further bent, causing the original strong chemical bonds to be distorted or deformed, and it is easier to break and generate small molecular substances with little or no pollution to the environment. At the same time, the formation of electric field increases the soil temperature, and part of the electric energy is transformed into heat energy, which accelerates the life cycle and activity of microorganisms. Under the action of potential difference, it is conducive to the diffusion of microorganisms, oxygen, nutrients and other particles in the soil, which all enhance the effect of remediation. In Experiment 6, Experiment 7 and Experiment 8, the electrodes were switched regularly to generate a non-uniform electric field acting on the contaminated soil, and the remediation effect was better than that of the experimental group without electrode switching. The effect of Experiment 8 was the best, and the degradation rate reached 67.5%. This may be because the electrode switching alleviates the extreme environment formed in the area near the electrode, keeps the pH of the soil in a relatively stable range, and the soil microorganisms near the electrode are stimulated by the electric field under the appropriate environment, which enhances the activity and accelerates the metabolism. Microorganisms relatively far away from the electrode also accelerate their growth under the action of electric field, and nutrients migrate non directionally to every corner of the soil under the action of non-uniform electric field, all of which accelerate the decomposition rate of oil.

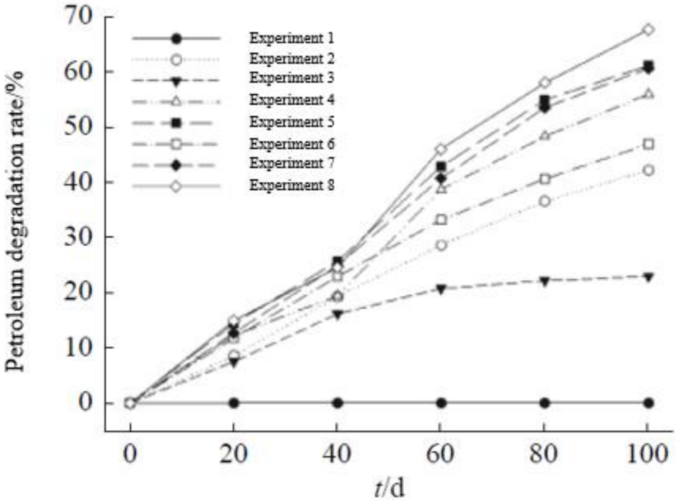

Figure 6. Petroleum degradation rate.

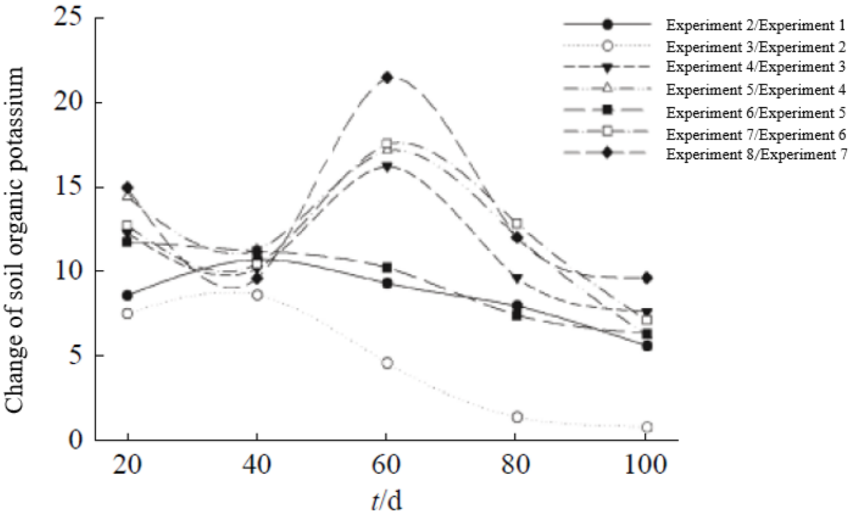

Figure 7. Ratio of Petroleum degradation rate.

Figure 7 shows the ratio of oil degradation rate every 20 days. It can be seen from the figure that the degradation rate of each group increased continuously at the initial stage of repair and decreased after 20 days. This is because after 20 days, the carbon source and other nutrients that are easy to be used by microorganisms in the soil are exhausted, resulting in a large number of deaths of microorganisms and a slow degradation rate. At 40 days, the degradation rate of Experiments 4, 5, 7 and 8 was significantly higher than that of other groups, and Experiments 4, 5, 7 and 8 were added with high-efficiency degradation bacteria and applied with electrokinetic remediation. The reason may be that the external electric field is applied to the soil continuously, so that the available N, P, K and other nutrients in the soil increase and accumulate to a certain amount, which can effectively meet the needs of microorganisms in the soil, accelerate the reproduction of soil microorganisms, and form the second degradation peak. It can be seen from the results that the combination of electrokinetic remediation and microbial remediation has a good effect on the remediation of oil contaminated soil. However, the research and understanding on the mechanism of electrokinetic microbial combined remediation is not very perfect, and needs further theoretical and experimental research.

3. Conclusion

Electrokinetic microbial remediation technology has a good effect on the remediation of oil contaminated soil. The electrode is switched once every 24 h, which effectively neutralizes the H+ and OH− produced by the anode and cathode, providing a new way for efficient degradation bacteria

A relatively stable living environment improves the degradation rate of oil. Regular electrode switching enables the non-directional migration of nutrients in the soil and dispersion to all parts of the soil, which can provide nutrients for more microorganisms to the greatest extent, prolong their life cycle, accelerate their reproduction speed, and improve the oil degradation rate. The applied electric field increased the contents of available N, P and K in soil to a certain extent, provided necessary nutrient elements for microorganisms to survive, and promoted the remediation effect of microorganisms. On the basis of electrokinetic microbial coupling remediation technology, the nutrients required by microorganisms were added to improve microbial activity and enhance the effect of electrokinetic remediation. The best oil degradation rate was 67.5%.

Funding: National key R & D plan (2020yfc-1808802); Supported by Shanghai local capacity building program (20090503200).

Conflict of interest: The authors declare no conflict of interest.

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