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# Enhancement of Bioremediation and Phytoremediation Using Electrokinetics

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Additional information is available at the end of the chapter

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## Abstract

This chapter discusses the use of bioremediation and phytoremediation coupled with electrokinetics and presents the elements contributing to the success of the remediation process. A deep discussion and an overview of the current advancement in the biotechnologies are outlined in details. Innovative solutions for challenges facing the field application of the new technology are presented and new directions are proposed. A careful review for contaminated site conditions including pH, temperature, and other factors influencing the behavior of microbial community are presented. Great deal of discussion is around overcoming the adverse effect of electrolysis reactions, which is a by-product of electrokinetics. The discussion includes prolonging the survival of the indigenous bacteria, increase of microbial enzyme secretion, improvement of the indigenous bacteria metabolism, and exploration of metagenomics resources from soil biota. The challenges facing the field application of bioremediation and phytoremediation including the delivery of the electron donors and/or acceptors and nutrients to microorganisms involved in the biodegradation, particularly in clay soils, which has very low hydraulic conductivity, is discussed. The use of electrokinetics in biostimulation application to enhanced degradation of organic pollutant is reviewed. The implementation of bioaugmentation in bioremediation coupled with electrokinetics to enhance the outcome of bioremediation is presented.

**Keywords:** bioremediation, phytoremediation, electrokinetic, biostimulation, bioaugmentation

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## 1. Introduction

In the last decade, different approaches have been used to mitigate soil contamination, however, many factors can affect the success of any remediation method including soil heterogeneity and

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the contaminant type. Researches and environmental engineers believe that there is no single remediation method that is suitable for every contaminated site; rather, an effective remediation program may involve the collective implementation of two or more methods [1]. Bioremediation is one of the most cost-effective remediation methods for contaminated soils [2]. Various bioremediation techniques have been used, with different degrees of success, to degrade pollutants at contaminated sites. Bioremediation types include biopile technique, landfarming approach, phytoremediation method, bioslurry, and bioventing.

Bioremediation employs microorganisms that have the ability to metabolize organic compounds including contaminants as food source in the soil. The traditional way to remediate contaminated sites typically depend on the type of soil and invariably involves “in situ” techniques like landfarming with occasional plowing or “ex situ” techniques such as windrows and biopile systems. In the former technique, the remediation process more or less relies on natural attenuation with minimal human input while in the latter cases, a great deal of human input and engineering is required to boost natural attenuation and accelerate remediation with minimal VOC emissions in the case of biopile systems. The literature covers a wide spectrum of approaches to soil remediation studies depending upon the discipline of the researchers: the treatment evaluation can be based on simple soil analysis for TPH, TOC (bulk parameters), or more sophisticated involving measurement of soil respiration rates and detailed chemical analysis of residual hydrocarbons in addition to the traditional bulk parameters. Indeed, recent studies indicate that relying on bulk parameters for the evaluation of the treatment process may still lead to highly hazardous residual petroleum hydrocarbon components [2, 3].

The success of a bioremediation process at specific site is mainly depend on the type of soil at the contaminated site, impermeable soil can exchange very little amount of oxygen and nutrients with the surrounding environment, therefore results on very slow remediation process. Microbiology area of research including microbial genomics, enzyme secretion, metabolism, and catalyst can be used to advance the knowledge in electrokinetic bioremediation. Bioremediation field applications are faced by obstacles such as the delivering of oxygen and nutrients to indigenous bacteria, particularly in soils with low hydraulic conductivity.

Electrokinetic remediation can be defined as the application of a low level direct current (DC) between a row of positively charged electrodes (anode) and negatively charged electrodes (cathode) placed at the edges of the soil under treatment [4]. The electric field incites three transport mechanisms, namely electroosmosis, electromigration, and electrophoresis, plus an electrolysis reaction at the electrodes. Electroosmotic flow is defined as the movement of water in the soil pores from anode to cathode under an applied electrical field. Electroosmotic flow (flow rate,  $q_A$  (m<sup>3</sup>/s)) can be calculated using an empirical formula similar to Darcy’s law of hydraulic flow rate:

$$q_A = k_e E A \quad (1)$$

where  $k_e$  (m<sup>2</sup>/s V) is coefficient of electroosmosis permeability,  $E$  (V/m) is the electric field intensity ( $-\frac{\Delta E}{\Delta L}$ ), and cross-section area,  $A$  (m<sup>2</sup>).

Electromigration is the transport of ions in the pores fluid toward the oppositely charged electrode. The migrational flux ( $J_m$ ) (the ionic movement toward the oppositely charged electrode in soil pore fluid) can be calculated by Acar and Alshawabkeh [4].

$$J_m = c_j u_j \Delta(-E) \quad (2)$$

where effective ion mobility,  $u_j$  ( $\text{m}^2/\text{s V}$ ), which is defined as the velocity of the ion in the soil under influence of a unit electric field gradient and can be evaluated as follows:

$$u_j = \frac{D_j z_j F}{RT} \tau n \quad (3)$$

where  $D_j$  ( $\text{m}^2/\text{s}$ ) is the diffusion coefficient of ion species  $j$  in dilute solution,  $z$  is the valence of ion species  $j$ ,  $F$  (96,487 C/mol) is the Faraday's constant,  $R$  (8.314 J/mol·K) is the universal gas constant,  $T$  (K) is the absolute temperature,  $\tau$  is the tortuosity factor, and  $n$  is the porosity of the soil.

Electrophoresis is the movement of charged colloids under an applied electrical field. Electrolysis reactions produce hydrogen ions at the anode and hydroxyl ions at the cathode [5]. The hydrogen ions lower the soil pH near the anode and form an acid front, while the hydroxyl ions increase the pH at the cathode vicinity generating a base front. The acid front travels from the anode to the cathode, whereas the base front moves from the cathode to the anode.

Oxidation reaction at the anode:



Reduction reaction at the cathode:



Electrokinetic remediation is a timely technology that can significantly enhance nutrients delivery to indigenous bacteria, thereby providing a tremendous potential for cleaning contaminated soils including fine-grained soils, which are usually difficult to cleanup using conventional methods [6–8]. Many studies have investigated the use of electrokinetics to improve the outcome of bioremediation [3]. The combination of electrochemical technology with bioremediation may promote the removal of metal ions that are often inhibitory to bacterial activity, thereby enabling complete remediation of the soil [9]. Unlike pressure-driven flows in which channeling of the fluid through the largest pores is inevitable, electrokinetics permits a more uniform flow distribution and a high degree of control over the direction of the flow [10, 11]. Transport phenomena associated with electrokinetics can be utilized to effectively deliver nutrients to indigenous bacteria in the soils, and to enhance bioavailability (electroosmotic flow can enhance desorption).

**Figure 1** shows the conceptual model where upon a release of petroleum hydrocarbon part of the contaminant evaporates and part moves through soil to contaminate subsurface. Depending on the site environmental conditions, indigenous microorganisms start to adapt and degrade

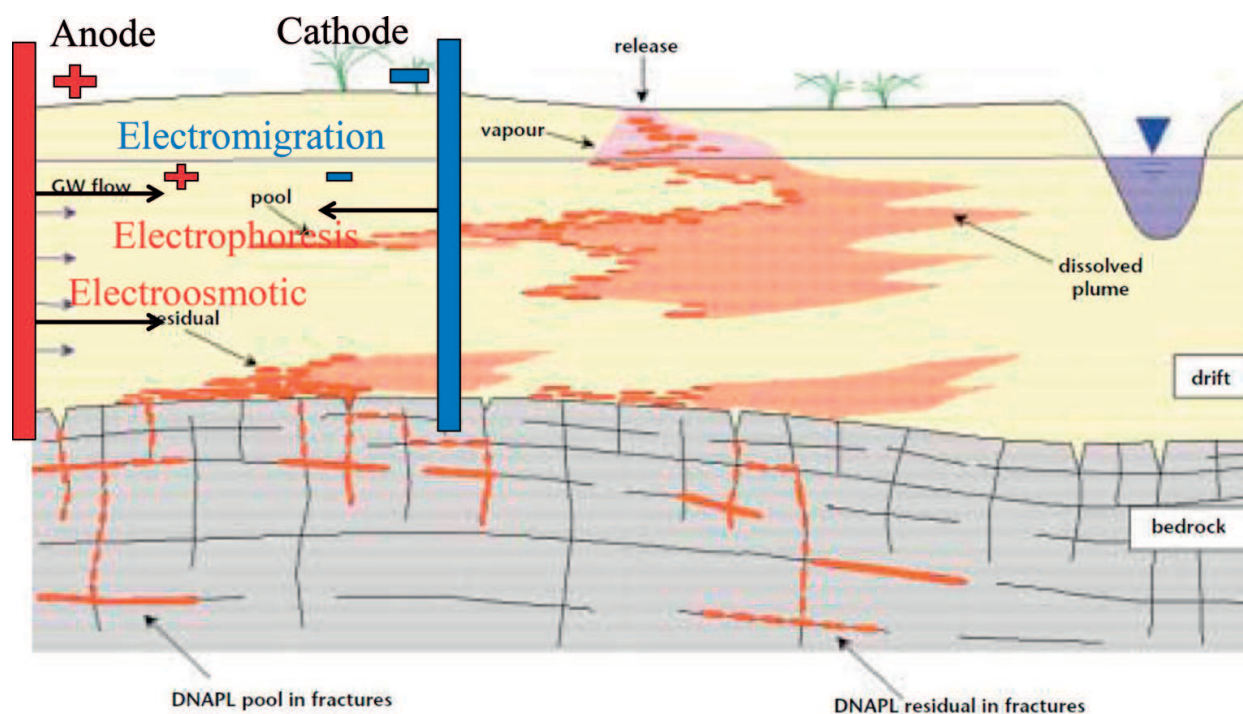


Figure 1. Conceptual model (after [43]).

contaminant. In most cases, the biodegradation rate is slow. Electrokinetics can be used to enhance the degradation rate by using the transport mechanisms associated with electrokinetics to deliver nutrients and/or to introduce new bacteria if the indigenous microorganisms are not capable of degrading the contaminant. However, the development of an acidic medium near the anode and an alkaline environment near the cathode by electrolysis reactions can create unfavorable condition for bacteria [7, 12, 13].

It is known that the application of the electric current during electrokinetic bioremediation increases the contaminated soil's temperature to high level which has an adverse effect on the survival of the indigenous bacteria. A recent study showed that the cost of electric power need for electrokinetic is a major part on the overall cost of electrokinetic remediation process. On the other hand, electricity power line may not be available in remotely located contaminated sites. Therefore, the energy consumption can increase the cost of the bioremediation process and result in restricting wide field applications of the electrokinetic bioremediation. Application of electrokinetic bioremediation can be divided into two main aspects:

- Microorganism-related factors, mainly how the environment at contaminated site can affect the degradation process.
- Electrokinetic processes; the phenomena associated with electrokinetic such as electrolysis reactions and the effect of other factors including the application of electric current, pH, soil temperature, and the availability alternatives concerning power source. In the following sections, each of the above-mentioned categories will be discussed.



## 2. Microorganisms-related factors

The environment conditions at contaminated site directly affect electrokinetic bioremediation process through supporting the existence of microorganisms, or enhancing bacterial viability and persistence in contaminated soil. The subsurface conditions and the characteristics of indigenous bacteria are the main factors affecting electrokinetic bioremediation process. Several factors can contribute to persistence of bacteria in the soil environment including pH, nutrients, electron acceptors, osmotic stress, and temperature [14, 15]. In addition to soil environment, the characteristics of the indigenous bacteria play a significant role in their existence, for example, some bacteria form biofilms which protects themselves from external stresses [16, 17]. Another mechanism for bacteria to survive is to produce spores [18]. In the event of severe weather and nutrient deprivation, bacteria will die eventually producing endospores which have a very hard shell that protects them [19]. Typically, under extremely poor living conditions, endospores are in a state of dormancy (sleeping condition); once the environmental conditions improve, the spore will germinate and outgrow [20]. The following techniques can be used to enhance bioremediation:

1. Genetic engineering can be used to enhance the outcome of bioremediation through changing or rewiring of microorganism metabolic pathways so as to enhance the ability of bacteria in degrading the targeted contaminant [21, 22]. Also, modifying bacterial genes and regulatory networks to make them tougher to survive and tolerate high contaminant concentration in soils [23].
2. Enhancing bioremediation using a commercially available enzyme which is currently very expensive, but the expected reduction in enzyme prices as the technology continues to improve can significantly reduce the initial cost [24, 25]. Previous studies showed that the use of enzyme in bioremediation is not feasible because of the existence of heavy metals and other compounds which can denature the enzymes. It was concluded that the use of enzyme in bioremediation is not recommended because the enzyme will be denature under the field conditions and will not last long. An alternative way would be the use of microbial genetics to clone/overexpress and introduce exogenous gene/gene clusters using as a host bacterial cell that will produce the required enzyme. The other challenge will be the delivery of the enzyme expressed in bacteria to the contaminated zone in order to degrade the pollutant [26]. A recent study showed that the use of genetically modified bacterial secretion system can enhance the bioremediation [27].
3. Exploring metagenomics to enhance electrokinetic bioremediation [28]. It is known that only 5% of the microorganisms in nature are culturable in the laboratories and can be studied. Microorganisms in nature can produce enzymes that are capable of degrading contaminants. The total genomic DNA can be collected from contaminated soil, then using the available technologies to slice the DNA into pieces, and clone it into a vector in order to form a library called 3D metagenomics DNA library. This library can be augmented into a selected bacterium that can be used to degrade contaminants providing that this library contains specific genes that can produce enzyme needed for the degradation process [29].

4. Characterizing the bacterial metabolism. It is very important to study the bacteria well before using the bacteria in the bioremediation process. In the contaminant degradation, knowing the degradation pathways are essential because some bacteria can produce secondary by-products/metabolites that are not in favor of the process. The knowledge of bacterial metabolism and the degradation pathways well can use to enhance the degradation by change/rewire bacterial metabolism to make them produce less/nontoxic products. More research is required in this area using the advancement in genetic engineering to eliminate/reduce the harmful by-products. Bacteria consortia at environmental sites communicate and coordinate behaviors and functionalities at community level using chemicals such as acyl homoserine lactones (AHLs), which is also described as bacterial quorum sensing (QS). In nature, QS may help bacteria for better and enhanced bioremediation. However, very few studies have investigated the role of QS in bioremediation [30–32].

### 3. Biostimulation and bioaugmentation

In bioremediation, there are three techniques that can be used at contaminated sites: (a) natural bioattenuation in which the pollutant is degraded by bacteria to nontoxic product, the process involve minimal to no human intervention; (b) biostimulation is defined as the introduction of supplements including nutrients, water, electron acceptors/donors to enhance the degradation process; and (c) bioaugmentation, which involves the use of large numbers of the bacterial strains that are capable of degrading the contaminant [33]. The following sections present the application of electrokinetics in biostimulation and bioaugmentation.

#### 3.1. Biostimulation

In bioremediation, bacteria is employed to degrade contaminants into nontoxic products, the success of the degradation process is mainly dependent on the growth and reproduction of bacteria. The growth and metabolisms of microorganisms are affected by the available amount of nutrients, electron acceptors/donors, and oxygen. Electroosmotic can be used to deliver nutrients and oxygen to indigenous bacteria in the contaminated zone. Moreover, electrokinetics can increase the opportunities of contact between bacteria and contaminants therefore, enhancing bioavailability. Previous studies discussed the use of electrokinetics in delivering nutrients are presented in **Table 1**. In a previous study, electrokinetics was used to deliver nutrients to contaminated zone under controlled pH conditions [34]. In this study, polarity exchange technique was used to control the soil pH. The result showed that high amount of contaminant was degraded under controlled pH conditions. Nitrate and ammonium were delivered to contaminated zone under uncontrolled pH conditions. The results showed that nitrate can be delivered successfully/precisely to the contaminated area near the anode while low amount of ammonium was transported to the cathode. More importantly, it has been observed that the nutrients were distributed evenly in the soil under controlled pH using the exchange polarity technique [35]. The results from electrokinetic bioremediation studies have shown that electrokinetics is successful in delivering nutrients to indigenous bacteria.

Soil	Voltage/current	Nutrient concentration	Highlights/main outcome	Reference
Clay loam	1 V/cm	2 g/L $\text{NH}_4\text{NO}_3$ 2 g/L $\text{KH}_2\text{PO}_4$	Nitrate transport rate 19 cm/d/v Phosphate results is not presented	[35]
Coarse sand	0.25 V/cm	1 g/L $\text{NaNO}_3$	Nitrate transported 0.6 cm/h	[41]
Clayey silt	0.5 V/cm	2 g/L $\text{NH}_4\text{NO}_3$ 5 g/L $\text{KH}_2\text{PO}_4$	Nitrate transport rate 5 cm/d/v Phosphate was not transported	[42]
Lean clay	0.85 V/cm	3.2 g/L $\text{NH}_4\text{OH}$ 0.48 $\text{H}_2\text{SO}_4$	400 mg/kg $\text{NH}_4\text{OH}$ 200 mg/kg $\text{H}_2\text{SO}_4$	[34]
Fine sand	15 $\mu\text{A}/\text{cm}^2$		Nitrate transported 250 mg/L	[1]
Kaolinite	123 $\mu\text{A}/\text{cm}^2$		Nitrate transported 250 mg/L	[1]

**Table 1.** Electrokinetic injections of nutrients.

However, excessive amounts of nutrients in soil exploit the growth and increase the intensity of microorganisms and consequently result in clogging the soil pores causing biofouling [34]. Therefore, it is important to study and carefully plan for the addition of nutrients. A recent study explored the possibility of providing oxygen to polluted soils by electrokinetics for aerobic bioremediation treatments of the soils. Oxygen ions, produced from the electrolysis reaction at the anode, are dissolved in water and transported by electroosmotic flow (see Eq. (1)). Due to the high porosity of the silty and sandy soils, the oxygen ions were transported inside the soil. It was observed that the dissolved oxygen concentrations were high (between 4 and 9 mg/L) in the contaminated zone. The presence of oxygen ions is essential for aerobic biodegradation processes. On the other hand, the transport was not possible in the clay soil.

### 3.2. Bioaugmentation

Bioaugmentation can be defined as the use of microorganisms which are capable of degrading contamination at specific site. There are two types of bioaugmentation: the first scenario is to increase the number of bacteria at contaminated site by introducing high number of colony forming units. In this case, the indigenous bacteria is capable of degrading the contaminant, however, the intensity of bacteria is low. In the second scenario, bacterial strain with superior capabilities is introduced to the contaminated site to enhance the degradation process because the indigenous bacterium is not capable of degrading the contaminant. Electroosmotic flow can be used to transport microorganism through contaminated soil to the contaminated zone [11]. For instance, the transport of bacteria in clay and sand by electroosmotic flow and electrophoresis was investigated. The results showed that 20% of bacteria were transported by electrophoresis. A more recent study showed that microorganisms can be transported by electrokinetics in sand via electrophoresis and the microorganisms remained active and viable after the transport process [36]. Another study showed that by adding bacteria in the anode and cathode compartment, bacteria was transported via electroosmotic flow in clay soil. However, in general, bioaugmentation studies have not been successful. The lack of success has been attributed to the formation of antibiotics by indigenous bacteria, predation and adaptability of new bacteria to the contaminated soil. For instance, *Pseudomonas* sp. LB400 bacteria were found to be capable of degrading 4-chlorobiphenyl in sterilized soil, but a



decrease in their viability was observed when non-sterilized soil was used. In many cases, there is a need for the use of more than one bacterial strain to be able to degrade contamination at specific contaminated site. Microbial consortia can degrade contamination with high efficiency, however, bacteria tend to compete with one another. There is an urgent need for a new technique that can minimize/eliminate the competition between the bacterial strains. In electrokinetic bioremediation, the application of electric current disrupts bacteria membrane by changing the orientation of membrane lipids. High current can be used to deactivate/kill bacterial strains that have negative impact in the degradation process. Application of high voltages in the range of  $25 \text{ kV cm}^{-1}$  per  $40 \text{ }\mu\text{s}$  pulse duration can kill bacteria. The effect of direct current application on different strains of bacteria in liquid and slurries has been investigated. Further research in this area is required to explore the use of electrokinetics as a tool to kill the unfavorable bacteria.

Enzymes can be used in bioremediation, instead of microorganism, to avoid the competition between the bacterial strains in the microbial consortia. The new technologies can be used to produce enzymes with high quality and increase the shelf time for the enzymes. Enzymes are simple to use in the bioremediation process and there is no unfavorable produce when using enzymes in the remediation process. The only down side of using enzyme is the high cost associated with the use of enzyme. The delivery of enzymes using electroosmotic flow has not yet been investigated.

## 4. Electrokinetic processes

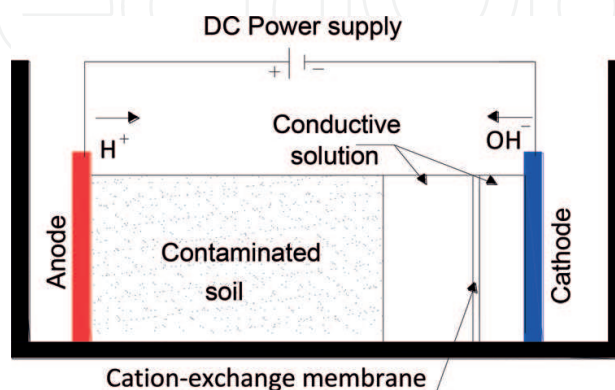
Application of electric field in electrokinetic remediation results in electrolysis reactions at the electrodes, electroosmotic flow from anode to cathode, electromigration of ions to the oppositely charged electrode, and electrophoresis. Aforementioned processes can change the physiochemical properties of the soil and subsurface conditions at the contaminated site. Electrolysis reaction results in acidic conditions near the anode and basic environment near the cathode. Application of electric current develops voltage gradients and forms zones with different current intensity. Soil temperature increases due to application of electric current. Contaminants attached to the soil matrix can be desorbed by the effect of electroosmotic flow and contribute to the bioavailability.

### 4.1. pH gradient

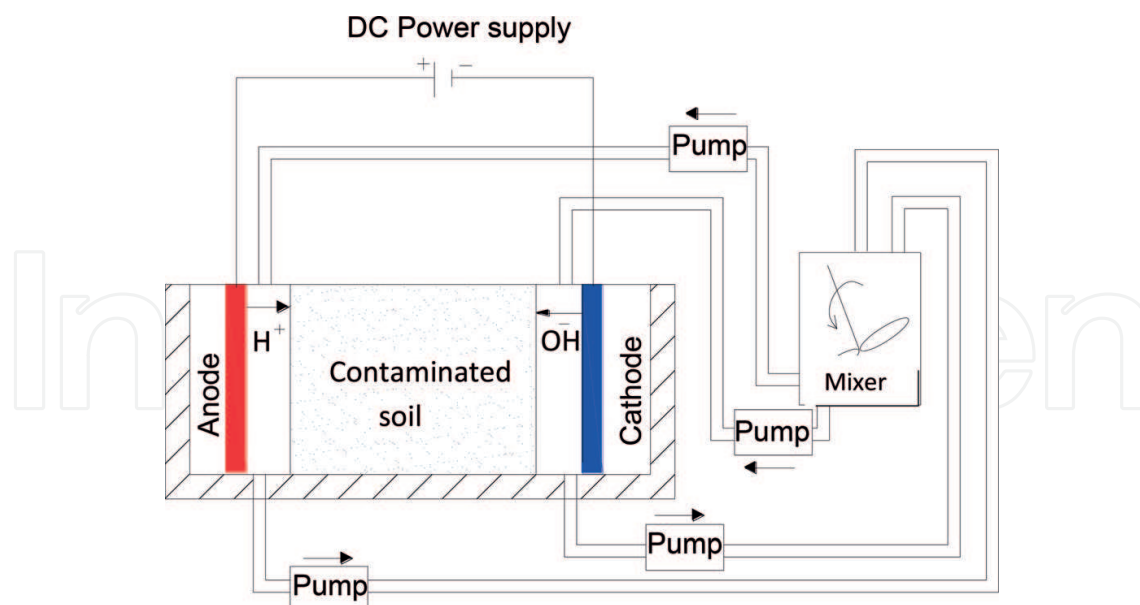
In electrokinetic processes, application of electric current results in occurrence of electrolysis reactions of water (redox) at the electrodes. Oxidation occurs at the positively charged electrode generating hydrogen ions and liberates oxygen gas. Reduction takes at the negatively charged electrode producing hydroxyl ions and hydrogen gas. The hydrogen ions (i.e.  $\text{H}^+$ ) form an acid front that travels toward the cathode by the effect of three mechanisms including: electroosmotic flow, diffusion, and electromigration. The acid front reduces the pH of the soil creating acidic environment. The hydroxide ions form a base front that travels toward the anode by electromigration and diffusion and elevate the pH of the soil in the vicinity of the

cathode. The base front is slower than the acid front because the base front moves in a direction that is against the electroosmotic flow and the hydroxyl ions ( $\text{OH}^-$ ) are heavier in weight than hydrogen ions ( $\text{H}^+$ ). Therefore the acid front and base front meets at a point closer to the cathode. The acidic environment near the anode and alkaline conditions at the vicinity of the cathode play a very important role in the electrokinetic processes. For example, the low pH conditions increase desorption of heavy metals from contaminated site. High and low pH environments have a crucial effect on the survival of bacteria at contaminated site. Heavy metals are soluble at low  $\text{pH} < 6$  and precipitate at a  $\text{pH} > 7$ . In electrokinetic remediation, the soil pH is in around 2–3.5 near the anode and between 8 and 11 at the cathode. For instance, cobalt and copper are tend to dissolve into solutions at pH around 4.5–6.5, and form insoluble hydroxides at pH greater than 7.5. The low pH near the anode contributes positively to the dissolution of heavy metals and enhances the removal process. On the other hand, high pH increases the precipitation of heavy metals in the vicinity of the cathode, and decreases the efficiency of the technique in removing contaminants. In electrokinetic bioremediation, the soil pH plays a dominant role in the success of the process. Bacterial survival and optimum degradation efficiency are directly related to the pH conditions. Bacteria can survive at a pH between 6 and 8. Some bacterial strains can tolerate very high and very low pH values. Bacteria have the capability of controlling the exchange of hydrogen ions through the cell wall to adapt the intracellular pH. However, it has been found that the high pH gradient across cell membrane has a detrimental effect on the growth and metabolism of bacteria.

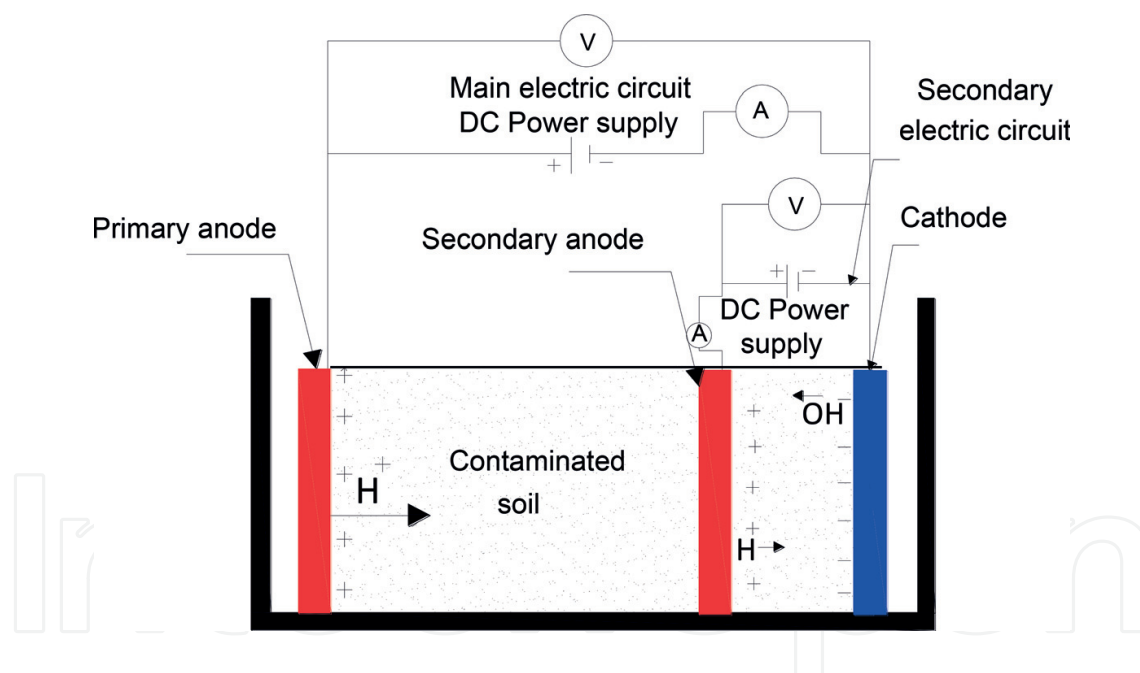
Researches and engineers have developed many techniques to control pH during electrokinetic remediation; these techniques can be divided into two approaches including conventional and innovative techniques. The conventional techniques include the ion selective membrane technique in which a cation-exchange membrane is introduced to cease the movement of the hydroxide ions from the cathode to the soil as shown in **Figure 2**; continuous changing/removing of the solution in the electrode compartments; and addition of chemical compounds such as ethylenediaminetetraacetic (EDTA), acetic acid, and nitric acid. On the other hand, innovative techniques include a stepwise moving anode, polarity exchange, circulation of an electrolyte (anolyte and catholyte) solution in the electrode compartments (see **Figure 3**), and the two anodes technique (TAT) (see **Figure 4**) which have investigated the control of the advancement of the acid and the base fronts [37]. There are many factors that can



**Figure 2.** Electrokinetic remediation with ion selective membrane (after [43]).



**Figure 3.** Electrokinetic remediation cell with electrolyte solution circulation technique (after [43]).



**Figure 4.** Two anode technique (TAT) (after [43]).

affect the soil pH including the soil type and the soil buffer capacity. The soil puffer capacity is directly influenced by the presence of anions, carbonates, hydrocarbonates, hydroxides, borates, phosphates, silicates, and organic acids anions. These factors should be taken into consideration before selecting the suitable technique to control the soil pH.

Many researchers have investigated the effect of pH on electrokinetic bioremediation using conventional methods. For instance, the use of electrokinetic bioremediation to mitigate

creosote-polluted clay soil was investigated. In this study, the soil pH was controlled by continuous substitution of the solutions used at the water compartments. The downside of using this technique is the amount of work involved in the process (removing/changing the solution) and the cost of the process plus this technique is not suitable for field applications. Moreover, in this technique it is required to treat the electrode solution (removed from the electrode compartment) before disposing it. The technique that involves the use of chemical compound is not recommended due to the possibility of generation of chemical reaction that has a negative impact in the remediation process. The addition of acids, such as hydrochloric acid and nitric acid, to reduce the pH near the cathode can result in acidic condition, which it is extremely difficult (if not impossible) to reverse its effect to the original condition [37]. The innovative techniques, that are available to control pH at contaminated sites, are expensive and in some cases are not applicable in field applications. For example, the step moving anode technique required labor work and involve the mobilization of the anode along the distance between the electrodes. Also, this technique is not suitable for electrokinetic bioremediation as it results in lowering the soil pH and that is not in the favor of the process. This technique is suitable for desorption and mobilization of heavy metals as the moving electrode (anode) generate hydrogen ions which result in lowering the soil pH ( $\text{pH} \leq 4.5$ ). The low pH environment is suitable for desorption of heavy metals from soil, while it affects bacterial survival in bioremediation processes.

In electrokinetic bioremediation, the low pH condition has adverse effect on indigenous bacteria. The measurement of pH during the polarity exchange technique is crucial for the success of the process. In a research study of phenol-contaminated soil, the polarity reversal technique was used to control the soil pH and water content. This technique can be suitable for electrokinetic bioremediation; however, continuous pH monitoring is required which is challenging and increases the overall cost of the process. In another research study, Kim et al. [34], developed and used a technique involves the circulation of electrolyte solution between the electrode compartments during the process as illustrated in **Figure 2**. This circulation of electrolyte solution can be used to control the pH during electrokinetic bioremediation; however, the issue will be the running cost and the need for maintenance in the field. The circulation of electrolyte solutions can be a challenging and very difficult for implementation in the field application. In electrokinetic bioremediation applications, the control of field conditions, especially pH, is very important for the survival of indigenous bacteria and contributes positively to the success of the process. Many techniques have been developed and implement to control pH during electrokinetics application. However, more research is required to enhance the outcome of the existing techniques. Also, there is a need for development of new techniques to control the pH during electrokinetic bioremediation application.

Recently, the authors of this chapter have investigated an innovative technique that can be used not only to stabilize pH but also to distribute nutrients uniformly inside the contaminated soil during electrokinetic bioremediation [37]. In the innovative technique, two electrodes are placed in each water compartment one serves as an anode and the other as a cathode to form two electric circuits that connected to a power supply. At each compartment, the anode will produce hydrogen ions and the cathode will produce hydroxyl ions, the coexistence of these

ions in the same compartment will result in neutralizing each other. As shown in Eqs. (4) and (5), the innovative configuration is supposed to forms the same numbers of hydrogen ions and hydroxide ions with all the ions reacting to form water. The innovative technique provides solutions for challenges facing other techniques; there is no need for continuous pumping or addition of chemical compounds.

4.2. Electric current density and voltage gradient

During electrokinetic bioremediation, microorganisms at contaminated site are subject to stress due to application of electric current. The electric current has direct and/or indirect effects on indigenous microorganisms. For instance, application of high voltage can cause a rupture in the cell membrane. Also, application of electric current can be a companied with chemical reactions that produce by-products which are harmful to the microorganisms. In the food industry, the electric current is used for disinfection purposes (killing bacteria). Studies showed that, the use of DC current causes the death/inactivation of living organisms. Over the last decade, researchers have investigated the influence of electric current on electrokinetic bioremediation treatment.

Microorganisms survival and transport during, electrokinetic remediation, is greatly affected by the application of electric current. **Table 2** presents data from previous studies that investigated the effect of electrical current on the indigenous bacteria. In a research study, the effect of

Medium	Current intensity or voltage gradient used	Highlights/main outcome	Reference
Liquid	20 mA/cm <sup>2</sup>	High cell density survive	[43]
Soil (kaolinite)	0.31, 0.63, 1.88, 3.13 mA/cm <sup>2</sup>	Optimum current 0.63 mA/cm <sup>2</sup>	[43]
Liquid	0.04, 4, 8, 12, 14 mA/cm <sup>2</sup>	Optimum electric field density 100 kJ/L	[41]
Liquid	10.2 mA/cm <sup>2</sup>	No effect on cell activity	[43]
Glass beads	1.8 mA/cm <sup>2</sup>	Low level DC has no effect of cell viability	[43]
Clay and silt	0.314 mA/cm <sup>2</sup>	pH changes near the anode is major factor affecting the microbial communities	[8]
Soil	1.0 mA/cm <sup>2</sup>	No negative effect on indigenes bacteria	[43]
Hide-soak liquors	2 A	Deactivated bacteria	[3]
Activated sludge	0.5–1.5 mA/cm <sup>2</sup>	pH or direct contact caused bacterial inhibition	[43]
Fine-grained soil	2 V/cm	The population of bacteria increased near the cathode	[43]
Sandy loam	0.46 V/cm	Rate of transport is 0.11 cm/h Microorganisms are active after the transport process	[3]
Tap water	0.28–1.4 V/cm	Optimum voltage intensity is between 0.28 and 1.4 V/cm	[8]

**Table 2.** Effects of electrical current.



electric current on the intensity of indigenous bacteria was investigated. It was concluded that the application of electric current is harmful on the microbial community with low cell densities; however, the electric current was minimal in microbial communities with high cell densities. Recent study, showed that the optimum degradation of an organic compounds (pentadecane in kaolinite soil) that the optimum pollutant removal was achieved using an intermediate electric current density occurs when using electric current intensity of  $0.63 \text{ mA/cm}^2$  compared with the higher and lower current densities of  $3.13$  and  $1.88 \text{ mA/cm}^2$ , respectively. Another study showed that selecting electric current intensity is essential not only in obtaining optimum degradation but also in retaining the indigenous microorganisms. This study showed that 37% of total petroleum hydrocarbons were degraded in the vicinity of the anode with an optimum electric field of  $2 \text{ V/cm}$ .

Previous studies showed that the use of electroosmotic and electromigration (electric field) to transport microorganisms did not reduce the capability of microorganism in degrading organic matter [36]. Very few studies investigated the effect of the electrode materials on the electrokinetic bioremediation. For instance, the results of an experimental study showed that indigenous microbial community is adversely affected by the products of the electrochemical reactions between the electrode material and the soil medium. The chemical reactions between the electrode material and the soil medium depend on many factors and it is very difficult to predict the by-products. It has been observed that not only the electric current intensity affects the microbial survival, but more importantly the combined effect of applied current intensity and duration is the crucial factor affecting living organisms. The use of steel, copper, and carbon as electrodes with different combination (anode-cathode) in electrokinetic remediation was investigated. The results showed that the efficiency of the remediation process is significantly affected by the selection of the electrode material and which material to be used in the anode and in the cathode. More research is needed in this area to investigate the effect of electrode materials in electrokinetic bioremediation.

### 4.3. Temperature

It is known that microorganisms can survive in various environmental conditions including a wide range of temperature. For instance, thermophile can tolerate temperature between  $45$  and  $120^\circ\text{C}$ , mesophile can live in temperature between  $20$  and  $45^\circ\text{C}$ , and psychrophile survive at low temperatures between  $-20$  and  $10^\circ\text{C}$ . In general, microorganisms' growth rate is directly proportional to the temperature between  $25$  and  $34^\circ\text{C}$ , also the increase in temperature results in increase of metabolism and the highest degradation occurs at temperature between  $27$  and  $42^\circ\text{C}$ . The increase in temperature during electrokinetic processes is reported in the available literature. For example, a recent study showed that application of electric current resulted in an increase in the soil temperature between  $5$  and  $20^\circ\text{C}$ , the maximum increase was observed in the vicinity of the anode. Another study showed that the temperature increased up to  $90^\circ\text{C}$  during field application and intermittent current was used to interrupt the electric current so as to reduce the temperature. Although, the increase in temperature during electrokinetic remediation is known and well documented, yet, very few reports in the current literature discussed the effect of temperature on electrokinetic bioremediation. Researchers tend to

attribute the increase in biodegradation to nutrient delivery by electrokinetic and over looked other factors that contribute positively to the process (i.e. temperature). In the current literature there are very few reports that studied the implication temperature in the electrokinetic bioremediation. In a previous study, electrokinetic was used to deliver nutrients and oxygen to microorganisms in the contaminated zone. It was concluded that the increase in soil temperature, resulted from application of electric current, enhanced the degradation process. The continuous application of electric current using high applied voltage for long period of time can increase the temperature inside the soil being treated. No doubt, the elevated temperature has a negative impact on the viability of indigenous bacteria. Intermittent current can be used to eliminate the effect of continuous current in increasing the soil temperature. The use of current intermittence can control the increase in temperature, and also can contribute positively to the outcome of the remediation process.

4.4. Bioavailability

Bioavailability can be measured by the amount of colony forming unit (CFU) of bacterial strains that capable of degrading the contaminants, the CFU must be available in soil liquid (pore fluid) at a given time. Bioavailability is also can be defined as the portion of contaminants that is available (ready) to be consumed by microorganisms without the need for desorption process (Table 3). When the contaminant come in contact with the soil, sorption of the pollutants by the soil will take place and the sorption rate is depending on environmental conditions including pH, temperature, etc. After sorption took place, the bioavailability of the contaminant will mainly be dependent on the back-diffusion process. Therefore, back-diffusion plays a dominant role in controlling the bioavailability of contaminants. The main two schools of thought concerning bioavailability are: (1) bacteria can degrade a contaminant regardless to the sorption and desorption process, even if it is attached to the soil matrix; (2) desorption of contaminants from soil is a prerequisite for the degradation process to occur (desorption of pollutants should take place first before microorganisms can degrade it). In electrokinetic remediation, electroosmotic flow creates flow net to the soil solids (within the double layer) therefore, electroosmotic flow can promote desorption of contaminants from soil matrix. In a previous study, the authors have compared the efficacy of electroosmotic flow and hydraulic

Medium	Contaminant	Contaminant concentration (mg/kg)	Highlights/Main outcome	Reference
Fine soil (from a contaminated site)	Petroleum hydrocarbon	78,600	37% reduction	[43]
Sand	Diesel	6800	60% reduction	[35]
Clayey soil	Phenanthrene	200	65% removal	[35]
Coarse sand/sand	Creosote	50, 200, 500, 900, 6800	50, 68, 80% reduction	[8]
Sandy loam	Phenol	180	58% reduction	[8]
Clay	Creosote	1300	35% reduction	[43]
Kaolinite	Pentadecane	1000, 5000, 10,000, 20,000	77.6% reduction	[34]

Table 3. Laboratory tests using different contaminants.

flow in promoting desorption of phenanthrene from clay soil [38]. The results showed that the concentration of phenanthrene in the effluents from the test conducted using electroosmotic flow are three to four times higher than the concentration in desorption test using hydraulic flow. Also, it was found that the energy consumed during the hydraulic flow tests was three orders of magnitude higher than the energy used during the electroosmotic flow tests.

#### 4.5. Available power sources for electrokinetics

Electrokinetic remediation requires electric power to apply voltage gradient between the electrodes, the energy consumption is considered as the factor number one that contributes to the total cost of the process. Therefore, the increase in energy cost results in an increase of the overall cost of the remediation process. No doubt the cost is one of the major factors affecting the selection of suitable remediation technique, thus high cost of a remediation process can be a major obstacle restricting wide field applications of this technology. Previous studies showed that the cost of energy represents 30% of the total cost of an electrokinetic remediation process; however, very few studies have discussed an alternative economical source of power that can reduce the cost of energy. Power generated by solar panels is renewable energy and has no negative impact on the environment.

Recently, the environmental awareness increases and the solar energy has become the center for the interest of scientists and the public as an environmentally friendly source of power. Recent report from Solar Buzz showed that more than 70% of the photovoltaic (PV) resources have been installed in northern hemisphere including countries such as Germany, Japan, USA, and Canada. Previous studies showed that the efficiency of the solar panel increased during the winter because of the cold weather. Although, power generated by solar panels can be an excellent candidate for power supply in electrokinetics, yet there are very few reports that have investigated the use of solar power as a source of power in electrokinetic bioremediation. The effect the off power period, during the night, on the remediation process is not presented in the literature. Some of the advantages of using solar panels to generate power for electrokinetic remediation are the elimination of the cost of electricity transmission and the reduction of power losses in the transmission lines. Solar panels produce direct current (DC) field that can be used in electrokinetic remediation without the need for a transformer. In the near future, it is expected that the solar cell prices will decrease and as the technology continues to improve that can significantly increase the efficiency of the solar panels. The power generated by solar panel fluctuates during the day (starts from zero before sun rise and increases until noon time and decreases to zero by the sun set) and it is directly dependent the weather conditions (sunny, cloudy, rainy, etc.). This can cause disruption in the power supply during the day and intervals of zero voltage at night, especially in the northern latitudes with little day light during winter.

The application electric field during electrokinetic remediation results in ions orientation in the pore fluid that resists electric current. The ions orientation reduce the efficiency of the remediation process, however, the interruption of the electric field allows the restoration of original ions orientation, which can enhance the remediation process. The non-stabilized electric current generated by solar panels can stimulate the remediation process by the effect of restoration of ions orientation as explained earlier. Many studies have proven that the use of current

intermittence can enhanced the outcome of an electrokinetic remediation. In a previous study, the authors have investigated the use of solar panels to generate power for the electrokinetic remediation to remediate soil contaminated with copper [39]. Three solar panels were used to generate electric field across the contaminated soil 41, 27 and 13.5 V. The results showed that the power generated by the solar panels was enough for mitigation of soil contaminated with heavy metals. In recent work, the authors used solar panels to generate power for the electrokinetic bioremediation to mitigate clay soil contaminated with phenanthrene. The results showed that solar panels can be used successfully to produce enough power for electrokinetic bioremediation of petroleum hydrocarbons. Moreover, the intervals of zero voltage at night can decrease soil temperature in field applications, which is a benefit.

## 5. Electrokinetic-enhanced phytoremediation of contaminated soils

Pollutants are released to the environment from anthropogenic activities and natural weathering processes. Human activities such as mining, industrial, and agricultural activities typically lead to contamination of soils by heavy metals and organic pollutants. Some heavy metals and PAH compounds have been identified as mutagenic, carcinogenic, and/or teratogenic contaminants.

Phytoremediation is a green and sustainable process in which plant species are used to remove, degrade, or sequester pollutants from soil or groundwater. Phytoremediation has been shown to be efficient and economically feasible for the treatment of large areas with low contaminant concentrations as compared to other remediation treatments. Both organic and inorganic contaminants can be removed or degraded by the growing plants by several mechanisms, namely; phytodegradation, rhizofiltration, phytoaccumulation, rhizodegradation, and phytostabilization. Phytodegradation is the breakdown of the absorbed organic chemicals by plant metabolic processes or by compounds produced by the plant. Phytodegradation of petroleum hydrocarbons was achieved in a soil that was also contaminated with heavy metals. Rhizofiltration is the removal of contaminants such as heavy metals and radionuclides in the soil by the root membranes [40]. Phytoaccumulation is the incorporation of inorganic chemicals such as heavy metals in plant tissues. Rhizodegradation is the degradation of organic contaminants near the root mass by bacteria and fungi stimulated by the root exudes and enzymes released by the plant. Recent study showed that phytoremediation with *Mirabilis Jalapa* L. removed 63% of total petroleum hydrocarbon from a contaminated soil in 127 days. Another study showed that *Medicago sativa* Linn and Fire Phoenix had removed 80–100% of eight PAH compounds from contaminated soils in 150 days. Phytostabilization is achieved by induced chemical changes to specific heavy metal contaminants causing the metal to become less bioavailable. For example, deep rooting plants could reduce the highly toxic Cr(VI) to Cr(III), which is much less soluble, that is, less bioavailable.

In the decade, the combination of phytoremediation and electrokinetic remediation has been proposed in an attempt to avoid the limitations of phytoremediation. Electrokinetic-enhanced phytoremediation technology consists of the application of a low intensity electric field to the contaminated soil in the close vicinity of growing plants. In this hybrid technology, the removal or



degradation of the contaminants is performed by the plant, whereas the electric field enhances the plant activity by increasing the bioavailability of the contaminants by desorption and transport of the contaminants. In recent research studies, electrokinetic phytoremediation technology with electric field applied between 8 and 24 h/day has shown promising results for the restoration of heavy metal contaminated soils. The use of electrokinetic-assisted phytoremediation for soils with hydrocarbon contaminants has not been reported in the literature.

## 6. Conclusion

Electrokinetic remediation has received more attention in recent years as a well-developed technique for in situ soil decontamination. The principles of electrokinetics have been recently explored to enhance bioremediation and phytoremediation treatment of contaminated sites. Electrokinetics requires low-level electric current which makes solar energy by lower end solar panels an excellent source of power for the technology. Solar energy is an innovative power alternative for electrokinetic-enhanced bioremediation/phytoremediation and can be economically viable, in particular for remote sites with no active power lines.

The electrokinetic bioremediation is a promising technique that can be used to mitigate contamination with organic and inorganic compounds. The main challenges facing the application of electrokinetic are the process cost and the pH gradient. The application of electric current results in high energy consumption that increases the overall cost of the remediation process. This is considered one of the major factors restricting the field application of the technology. Very few studies in the current literature have addressed the cost of energy in electrokinetic bioremediation. Research to date has shown that electrokinetic bioremediation can be used to mitigate contaminated soil, however, the results showed a low to moderate percentage of contaminant degradation. Future research is needed to address the optimization and the removal efficiency of the electrokinetic bioremediation. Researchers and engineers address the pH issue using two different approaches conventional and innovative techniques. The conventional techniques involve the addition of chemical compounds to control the pH. The innovative techniques involve circulation/mixing of the electrolyte solution at the anode and cathode compartment to neutralize the pH. Both conventional and innovative techniques can result in a further increase in the overall cost of remediation process. Moreover, the effect of the increase in temperature associated with electrokinetic bioremediation has not been fully investigated.

The recent advancement in biotechnology and bioengineering provides incredible opportunities for enhancing electrokinetic bioremediation and phytoremediation. The application of electrokinetic bioremediation/phytoremediation involves various microorganisms aspects including the challenges facing the microbes survival in contaminated sites; isolation and characterization of bacterial strains with superior degradation capabilities in bioremediation; the improvement of the metabolism of microorganisms through manipulation using genetic engineering; identifying powerful degrading enzymes using metagenomics; and exploring new engineering ways to enhance bioremediation.

It is anticipated that, with intense research effort, the promising potential of the hybrid electrokinetic bioremediation/phytoremediation technology will encourage further research in



order to optimize the efficiency and expedite the transfer of the technology for in situ applications. Carefully designed, simulated and monitored laboratory and field studies are necessary to explore the fundamental and practical aspects of the technology. It is important to simulate and test different electrode configurations and identify hyperaccumulator plants of heavy metals and PAH compounds. It is also necessary to understand the influence of electric field in the physiology of the plant and soil microflora as well as the geobiochemistry involved in the degradation of organics in the rhizosphere.

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## References

- [1] Acar YB, Rabbi MF, Ozsu EE. Electrokinetic injection of ammonium and sulfate ions into sand and kaolinite beds. *Journal of Geotechnical and Geoenvironmental Engineering*. 1997;**123**(3):239-249. DOI: 10.1061/(ASCE)1090-0241
- [2] Chen M, Xu P, Zeng G, Yang C, Huang D, Zhang J. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting. Applications, microbes and future research needs. *Biotechnology Advances*. 2015;**33**(6 pt 1):745-755. DOI: 10.1016/j.biotechadv.2015.05.003
- [3] Yeung AT, Gu YY. A review on techniques to enhance electrochemical remediation of contaminated soils. *Journal of Hazardous Materials*. 2015;**195**:11-29. DOI: 10.1016/j.jhazmat.2011.08.047
- [4] Acar YB, Alshawabkeh AN. Principles of electrokinetic remediation. *Environmental Science & Technology*. 1993;**27**(13):2638-2647. DOI: 10.1021/es00049a002
- [5] Reed BE, Berg MT, Thompson JC, Hatfield JH. Chemical conditioning of electrode reservoirs during electrokinetic soil flushing of Pb-contaminated silt loam. *Journal of Environmental Engineering—ASCE*. 1995;**121**(11):805-815. DOI: 10.1061/(ASCE)0733-9372(1995)121:11(805)
- [6] Yeung AT, Corapcioglu M. Electrokinetic flow processes in porous media and their applications. *Advances in Porous Media*. 1994;**2**:309-395

- [7] Alshawabkeh AN. Electrokinetic soil remediation: Challenges and opportunities. *Separation Science and Technology*. 2009;**44**(10):2171-2187. DOI: 10.1080/01496390902976681
- [8] Reddy KR, Cameselle C. *Electrochemical Remediation Technologies for Polluted Soils, Sediments and Groundwater*. Hoboken, NJ: Wiley; 2009. 732 p. ISBN: 978-0-470-38343-8
- [9] Chilingar GV, Loo WW, Khilyuk LF, Katz SA. Electrobioremediation of soils contaminated with hydrocarbons and metals: Progress report. *Energy Sources*. 1997;**19**(2):129-146. ISBN: 978-0-471-73778-0
- [10] Shapiro AP, Probstein RF. Removal of contaminants from saturated clay by electroosmosis. *Environmental Science & Technology*. 1993;**27**(2):283-291. DOI: 10.1021/es00039a007
- [11] DeFlaun MF, Condee CW. Electrokinetic transport of bacteria. *Journal of Hazardous Materials*. 1997;**55**(1-3):263-277. DOI: 10.1016/S0304-3894(97)00023-X
- [12] Nyer EK, Suarez G. Treatment technology: In situ biodegradation is better than monitored natural attenuation. *Ground Water Monitoring and Remediation*. 2002;**22**(1):30-39. DOI: 10.1111/j.1745-6592.2002.tb00651.x
- [13] Luo QS, Zhang XH, Wang H, Qian Y. The use of non-uniform electrokinetics to enhance in situ bioremediation of phenol-contaminated soil. *Journal of Hazardous Materials*. 2005; **121**(1-3):187-194. DOI: 10.1016/j.jhazmat.2005.02.007
- [14] Inglis TJJ, Sagripanti JL. Environmental factors that affect the survival and persistence of *Burkholderia pseudomallei*. *Applied and Environmental Microbiology*. 2006;**72**(11):6865-6875. DOI: 10.1128/AEM.01036-06
- [15] Locatelli A, Spor A, Jolivet C, Piveteau P, Hartmann A. Biotic and abiotic soil properties influence survival of *Listeria monocytogenes* in soil. *PLoS One*. 2013;**8**(10):e75969. DOI: 10.1371/journal.pone.0075969
- [16] Flemming H-C, Wingender J, Szewzyk U. *Biofilm Highlights*. Dordrecht: Springer; 2011. 243 p. DOI: 10.1007/978-3-642-19940-0
- [17] Malik A, Grohmann E, SpringerLink. *Environmental Protection Strategies for Sustainable Development*. Dordrecht: Springer; 2012. 591 p. DOI: 10.107/978-94-007-1591-2\_1
- [18] Maldonado LA, Quintana ET. Unexpected properties of micromonosporae from marine origin. *Advances in Microbiology*. 2015;**5**:452-456. DOI: 10.4236/aim.2015.56046
- [19] McKenney PT, Driks A, Eichenberger P. The *Bacillus subtilis* endospore: Assembly and functions of the multilayered coat. *Nature Reviews Microbiology*. 2013;**11**(1):33-44. DOI: 10.1038/nrmicro2921
- [20] Abel-Santos E. *Bacterial Spores: Current Research and Applications*. Norfolk, UK: Caister Academic Press; 2012. DOI: 10.1086/675034
- [21] Qin JF et al. Modular pathway rewiring of *Saccharomyces cerevisiae* enables high-level production of L-ornithine. *Nature Communications*. 2015;**6**:8224. DOI: 10.1038/ncomms9224

- [22] Sah S, Aluri S, Rex K, Varshney U. One-carbon metabolic pathway rewiring in *Escherichia coli* reveals an evolutionary advantage of 10-formyltetrahydrofolate synthetase (Fhs) in survival under hypoxia. *Journal of Bacteriology*. 2015;**197**(4):717-726. DOI: 10.1128/JB.02365-14
- [23] Kang A, Chang MW. Identification and reconstitution of genetic regulatory networks for improved microbial tolerance to isooctane. *Molecular Biosystems*. 2012;**8**(4):1350-1358. DOI: 10.1039/C2MB05441H
- [24] McErlean C, Marchant R, Banat IM. An evaluation of soil colonisation potential of selected fungi and their production of ligninolytic enzymes for use in soil bioremediation applications. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*. 2006;**90**(2):147-158. DOI: 10.1007/s10482-006-9069-7
- [25] Karigar CS, Rao SS. Role of microbial enzymes in the bioremediation of pollutants: A review. *Enzyme Research*. 2011;(2001):1-11. DOI: 10.4061/2011/805187
- [26] Brenner K, You LC, Arnold FH. Engineering microbial consortia: A new frontier in synthetic biology. *Trends in Biotechnology*. 2008;**26**(9):483-489. DOI: 10.1016/j.tibtech.2008.05.004
- [27] Schneewind O, Missiakas DM. Protein secretion and surface display in Gram-positive bacteria. *Philosophical Transactions of the Royal Society B-Biological Sciences*. 2012;**367**(1592):1123-1139. DOI: 10.1098/rstb.2011.0210
- [28] Ray D, Mohandass SN, Roy P. Molecular phylogeny of arctic microbes using metagenomic approach. *Advances in Microbiology*. 2014;**4**:1278-1284. DOI: 10.4236/aim.2014.416137
- [29] Yergeau E, Sanschagrin S, Beaumier D, Greer CW. Metagenomic analysis of the bioremediation of diesel-contaminated Canadian high arctic soils. *PLoS One*. 2012;**7**(1):e30058. DOI: 10.1371/journal.pone.0030058
- [30] Miller MB, Bassler BL. Quorum sensing in bacteria. *Annual Review of Microbiology*. 2001;**55**:165-199. DOI: 10.1146/annurev.micro.55.1.165
- [31] Rutherford ST, Bassler BL. Bacterial quorum sensing: Its role in virulence and possibilities for its control. *Cold Spring Harbor Perspectives in Medicine*. 2012;**2**(11):pii (1-25)a012427. DOI: 10.1101/cshperspect.a012427
- [32] Huang YL, Zeng YH, Yu ZL, Zhang J, Feng H, Lin XC. In silico and experimental methods revealed highly diverse bacteria with quorum sensing and aromatics biodegradation systems—A potential broad application on bioremediation. *Bioresource Technology*. 2013;**148**:311-316. DOI: 10.1016/j.biortech.2013.08.155
- [33] Madsen EL. Determining in situ biodegradation—Facts and challenges. *Environmental Science & Technology*. 1991;**25**(10):1663-1673. DOI: 10.1021/es00022a001
- [34] Kim SJ, Park JY, Lee YJ, Lee JY, Yang JW. Application of a new electrolyte circulation method for the ex situ electrokinetic bioremediation of a laboratory-prepared pentadecane contaminated kaolinite. *Journal of Hazardous Materials*. 2005;**118**(1-3):171-176. DOI: 10.1016/j.jhazmat.2004.10.016

- [35] Xu W, Wang CP, Liu HB, Zhang ZY, Sun HW. A laboratory feasibility study on a new electrokinetic nutrient injection pattern and bioremediation of phenanthrene in a clayey soil. *Journal of Hazardous Materials*. 2010;**184**(1–3):798-804. DOI: 10.1016/j.jhazmat.2010.08.111
- [36] Maillacheruvu KY, Chinchoud PR. Electrokinetic transport of aerobic microorganisms under low-strength electric fields. *Journal of Environmental Science and Health Part A—Toxic/Hazardous Substances & Environmental Engineering*. 2011;**46**(6):589-595. DOI: 10.1080/10934529.2011.562832
- [37] Hassan I, Mohamedelhassan E, Yanful EK, Bo MW. A new technique for enhancing electrokinetic bioremediation. *Environmental Geotechnics*. 2016. E-ISSN 2051-803X. DOI: 10.1680/jenge.16.00001
- [38] Hassan I, Mohamedelhassan E, Yanful EK, Yuan Z-C. Sorption of phenanthrene by kaolin and efficacy of hydraulic versus electroosmotic flow to stimulate desorption. *Journal of Environmental Chemical Engineering*. 2015;**3**(4, Part A):2301-2310. DOI: 10.1016/j.jece.2015.08.011
- [39] Hassan I, Mohamedelhassan E, Yanful EK. Solar powered electrokinetic remediation of Cu polluted soil using a novel anode configuration. *Electrochimica Acta*. 2015b;**181**:58-67. DOI: 10.1016/j.electacta.2015.02.216
- [40] Lee M, Yang M. Rhizofiltration using sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. *vulgaris*) to remediate uranium contaminated groundwater. *Journal of Hazardous Materials*. 2010;**173**(1–3):589-596. DOI: 10.1016/j.jhazmat.2009.08.127
- [41] Tiehm A, Augenstein T, Ilieva D, Schell H, Weidlich C, Mangold KM. Bio-electro-remediation: Electrokinetic transport of nitrate in a flow-through system for enhanced toluene biodegradation. *Journal of Applied Electrochemistry*. 2010;**40**(6):1263-1268. DOI: 10.1007/s10800-010-0101-2
- [42] Schmidt CAB, Barbosa MC, de Almeida MDS. A laboratory feasibility study on electrokinetic injection of nutrients on an organic, tropical, clayey soil. *Journal of Hazardous Materials*. 2007;**143**(3):655-661. DOI: 10.1016/j.jhazmat.2007.01.009
- [43] Hassan I, Mohamedelhassan E, Yanful EK, Yuan Z-C. A review article: Electrokinetic bioremediation current knowledge and new prospects. *Advances in Microbiology*. 2016;**6**(1):57-75. DOI: 10.4236/aim.2016.61006

