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A Review Article: Electrokinetic Bioremediation Current Knowledge and New Prospects

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Abstract

This study discusses factors affecting various processes involved in bioremediation coupled with electrokinetics. The study presents innovative solutions, and proposes new directions. Environmental conditions that have an influence on the characteristics, behavior, and metabolism of indigenous microorganisms are presented. The discussion focuses on overcoming the unfavorable conditions created by electrolysis reactions, prolongation the survival of the microbes at contaminated sites, increase of microbial enzyme secretion, improvement of the indigenous bacteria metabolic pathways, and exploration of metagenomics resources from soil biota. The challenge facing the implementation of conventional bioremediation techniques in precisely and effectively delivering nutrients to indigenous bacteria, particularly in soils with tortuous paths and low hydraulic conductivity is discussed. Current knowledge in application of enhanced biostimulation using electrokinetics is reviewed. The implementation of bioaugmentation in bioremediation coupled with electrokinetics to enhance the outcome of bioremediation is presented. Effects of phenomena associated with electrokinetics in the hybrid remediation approach are discussed.

Keywords

Bioremediation, Bioaugmentation, Biostimulation, Electokinetics, Contamination

1. Introduction

Globally, fossil fuels are among the most important energy sources. Despite efforts to replace fossil fuels with

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renewable energy sources, it is expected that the market will continue to rely on fossil fuels over the next few decades. Crude oil drilling, petroleum extraction, and petroleum products delivery by pipelines, rail and tanker trucks inevitably cause oil spills on land, which creates serious environmental problems. Therefore, it is urgently necessary to develop innovative and cost-effective technology for the removal of petroleum hydrocarbons from contaminated soil. In fact, due to the large amount of accidental oil spills that have occurred frequently, soil or land remediation becomes more important than ever. A report by Canadian Council of Ministers of the Environment [1] indicates that 60% of contaminated site in Canada involve petroleum hydrocarbons. A report by Alberta's government (Alberta, Canada) showed that approximately 29,000 spills occurred between 1975 and 2013. Alberta has averaged two oil spills per day for the past 37 years [2].

Over the years, various remediation methods have been used with varying degrees of success to mitigate soil contamination. Due to soil heterogeneity and the diverse nature of contamination, the scientific community believes that there will not be a single universal remediation method suitable for all types of soils and pollutants; instead, an effective remediation program may involve the collective implementation of two or more methods [3]. Bioremediation is one of the most cost-effective remediation methods for contaminated soils [4] [5]. There are various bioremediation techniques, including biopile, landfarming, phytoremediation, bioslurry, and bioventing that can be used to degrade pollutants at contaminated sites. Environmental microorganisms, in particular, bacteria, are ubiquitous in nature. Indigenous bacteria have naturally evolved ability to metabolize diverse chemicals including pollutants as food source in the soil. This provides a great opportunity to utilize such bacteria in the cleanup of contaminated lands. From the biological and chemical points of view, bioremediation is the employment of indigenous bacteria present in a contaminated environment to degrade pollutants [4] [5].

The type of soil in a contaminated site usually plays an important role in the effectiveness of bioremediation. Current knowledge and advancements in microbiology, such as microbial genomics, metabolism, catalyst, microbial community or soil microbiome, and enzyme secretion can be further manipulated, designed and optimized to enhance the outcome of electrokinetic bioremediation. The main challenge facing the implementation of *in-situ* conventional bioremediation techniques is the difficulty of effectively and precisely delivering nutrients to indigenous bacteria, particularly in soils with low hydraulic conductivity. 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 [9]. 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 [10]. 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, namely, electroosmotic flow, electromigration, and electrophoresis, can be utilized to effectively deliver nutrients to indigenous bacteria in the soils, and to enhance bioavailability (electroosmotic flow can enhance desorption). 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 [6] [12] [13]. In addition, electric current and the associated increase in temperature may affect the bacteria survival during the bioremediation process. Energy consumption is also a major component of the total expenditure of electrokinetic remediation, and sometimes electricity power may not be available in remote areas. Energy consumption increases the overall cost of the bioremediation process and can become a major obstacle restricting wide field applications of this technology. This paper presents the current knowledge, discusses the major challenges facing field applications of electrokinetic bioremediation, identifies gaps in knowledge, and proposes key research frontiers and areas that future research needs to address.

Electrokinetic bioremediation processes can be divided into two main aspects:

- Microorganism related factors such as the existence of nutrients, and the microorganisms' capability of surviving, persisting, and degrading the contaminant.
- Electrokinetic processes; influence of electrokinetic processes include electrolysis reactions, electric current, change in temperature, power for electrokinetics, the availability of power lines near the contamination sites and the cost of electricity.

In the following sections, each of the above-mentioned categories will be discussed.

2. Microorganisms Related Factors

There are several aspects concerning bacteria or microbes in enhancing the outcome of electrokinetics bioremediation through prolonging the survival of the microbes in soil, or improving bacterial viability and persistence in contaminated soil. This can be divided into two main factors: the soil environment and the characteristics of indigenous bacteria. Survival and persistence of bacteria in the soil environment is affected by changes in soil pH, osmotic stress, temperature (cold or hot weather), UV exposure and chemicals [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 and protect them [19]. Typically, under extremely poor living conditions, endspores are in a state of dormancy (sleeping condition); once the environmental conditions improve, the spore will germinate and outgrow [20].

There are several strategies that can be explored to manipulate microbes to augment and boost bioremediation as discussed below.

1) Exploring the indigenous bacteria metabolic pathways and using genetic engineering to change or rewire bacterial metabolic pathways to strengthen microorganisms' ability for bioremediation [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]. At contaminated sites, many heavy metals and other chemicals present in soil will denature enzymes which are essentially proteins. Therefore, it will be impractical to use the enzyme directly in bioremediation because such enzyme will be inactivated in the actual soil environment and will not last long. However, microbial genetics can be used to clone, introduce and overexpress exogenous gene or gene clusters in a host bacterial cell so that the bacteria will gain extra ability to continuously produce the enzyme required for bioremediation. However, the enzyme expressed in bacteria has to be delivered or secreted by the bacteria to the environment in order to degrade the contaminant [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]. Microbes are a rich source of enzymes and products such as antibiotics. Nevertheless, only less than 5% microbes in nature are culturable and studied so far. Most of these microbes are not culturable in laboratories. Scientists and researchers can harvest the total genomic DNA from soil samples, then chip down the total soil DNA into smaller pieces, and clone it into a vector to make a library. Such a library is called 3D metagenomics DNA library. The cloned library can be introduced into a host bacterium. If such a DNA library contains useful genes or gene clusters that express enzyme capable of degrading contaminants, host bacterium will become more powerful in bioremediation [29].

4) Characterizing the bacterial metabolism well prior to application for biomediation. One issue limiting bioremediation is the bacterial production of secondary metabolites that can be more toxic or harmful than the original source contaminants. If we understand bacterial metabolism and the underlying regulatory pathways well, we can change and rewire its metabolism to make them produce less or no toxic products. This area needs to be investigated using the available genetic engineering to reduce the undesired metabolites and enhance the bioremediation outcome.

5) Investigating bacterial activity at the community level, *i.e.*, microbiomes, at contaminated sites. Bacteria rely on some nutrients to enhance their ability at degrading contaminants. Some nutrients or chemical compounds stimulate bacterial growth or promote the growth of bacteria cocktails and survive better at the community level. 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). QS is well studied for bacterial pathogens as QS regulate bacterial genes and function at the community level so that bacteria collectively act or infect host together, so QS coordinates infection. In addition, bacterial QS can be interfered by chemicals produced by host organisms or already available in nature [21] [22]. In nature, QS may help bacteria for better and enhanced bioremediation. However, very few studies have investigated the role of QS in bioremediation [30].

6) Isolating and identifying new bacterial strains from nature with enhanced bioremediation abilities, which are resistant to soil and environmental stress conditions and very efficient in bioremediation. The limitation for

such isolation is that sometimes these isolates cannot grow in synthetic medium in the laboratory. Therefore, modified medium recipe should be used to allow such bacteria to grow. However, it is really a low chance event, as it is not known which nutrients and components are required to support the growth of such bacteria. The authors have isolated and characterized dozens of bacterial strains from soil according to their ability to degrade diesel efficiently. Three strains have been selected and subjected to further investigation, including identifying functional genes, the ability to grow at different temperatures and pH. This area should be the focus of future research to meet the challenge of bioremediation. In addition, diversifying the samples to isolate such bacteria, *i.e.*, not just focusing on soil, but taking, for instance, samples from the bottom of the sea, forest, hot spring water, or oil field or oil refinery plant may lead to the discovery of novel strains.

3. Biostimulation and Bioaugmentation

Generally speaking, there are three treatment strategies employed for in situ bioremediation: a) natural bioattenuation where the contaminant is transformed to a lesser harmful product, b) biostimulation in which the biodegradation is accelerated by the addition of nutrients, water, electron acceptors or donors, and c) bioaugmentation, which involves the addition of genetically engineered microorganisms or microorganisms with enhanced degradation capabilities to the contaminated zone [31]. In the following sections the implementation of electrokinetics in biostimulation and bioaugmentation is discussed.

3.1. Biostimulation

As bioremediation uses microorganisms to degrade pollutants to harmless products, the success of the technique depends on the growth and reproduction of bacteria. Nutrients and often oxygen are necessary to stimulate the growth and metabolisms of microorganisms. Electrokinetics transports and controls the direction of ion movement inside soil. Therefore, when combined with bioremediation, electrokinetics can deliver nutrients to indigenous bacteria in the soil and increase mixing between bacteria and contaminants. **Table 1** shows some studies that investigated the delivery of nutrients using electrokinetics. The use of electrokinetics for nutrients delivered under controlled pH conditions was investigated [32]. The results showed higher contaminant removal when a polarity exchange technique (bidirectional) is used to deliver the nutrients compared with one direction electric fields (conventional). Under uncontrolled pH environments, the feasibility of effectively transporting two common microorganism nutrients (nitrate and ammonium) by electrokinetics was demonstrated [33]. The results showed that a high amount of nitrate was successfully transported to the anode compared with the ammonium transported to the cathode. The use of the exchange polarity technique to controlled pH resulted in an even distribution of nutrients in the soil compared with one direction electric fields [34]. The results from EK bioremediation studies have shown that EK is successful in delivering nutrients to indigenous bacteria. However, excessive amounts of nutrients in soil exploit the growth and increase the intensity of microorganisms and consequently

Table 1. Electrokinetic injections of nutrients.

Soil	Voltage gradient and electrical current	Nutrient concentration	Highlights/main outcome	Reference
Clay loam	1 V/cm	2 g/L NH_4NO_3 2 g/L KH_2PO_4	Nitrate transport rate 19 cm/d/v phosphate results is not presented	[34]
Coarse sand	0.25 V/cm	1 g/L $NaNO_3$	Nitrate transported 0.6 cm/h	[37]
Clayey silt	0.5 V/cm	2 g/L NH_4NO_3 5 g/L KH_2PO_4	Nitrate transport rate 5 cm/d/v Phosphate was not transported	[33]
Kaolinite Lean clay (CL)	0.85 V/cm	3.2 g/L NH_4OH 0.48 H_2SO_4	400 mg/kg NH_4OH 200 mg/kg H_2SO_4	[35]
Fine sand	15 µA/cm^2		Nitrate transported 250 mg/L	[38]
Kaolinite	123 µA/cm^2		Nitrate transported 250 mg/L	[38]
Fine soil sandy-clay	0.5 V/cm	1 g/L NH_4NO_3	Nitrate transported 1.5 mg/kg	[39]
Kaolinite	0.4 V/cm	50 mg/L NO_3-N 50 mg/L PO_4-P	Nitrate transported Phosphorus was not transported	[40]

result in clogging the soil pores causing biofouling [35]. 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 [36]. The transported oxygen was generated by electrolysis reaction of water at the anode (see Equation (1)). The results showed that oxygen transport occurred in the silty and sandy soils obtaining high dissolved oxygen concentrations between 4 and 9 mg/L which are useful for aerobic biodegradation processes, while transport was not possible in the clay soil.

3.2. Bioaugmentation

Introducing new strains of bacteria (bioaugmentation) with superior degradation capabilities can enhance bioremediation outcome. Many researchers have used phenomena associated with electrokinetics to deliver microorganism to contaminated soil. For instance, the transport of bacteria in clay and sand by electroosmotic flow and electrophoresis was investigated [41]. The results showed that 20% of bacteria were transported by electrophoresis. 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 [42]. Another study showed that by adding bacteria in the anode and cathode compartment bacteria was transported via electroosmotic flow in clay soil [43]. 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 [44] [45]. 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 [46] [47]. Many studies have suggested the use of microbial consortia to mitigate contaminated sites. It is generally known that microbial species do compete one another. Recent reports in microbiology have highlighted the need for an innovative technology that can be used to get rid of or reintroduce certain strains of bacteria [26]. In electrokinetic bioremediation, the application of electric current disrupts bacteria membrane by changing the orientation of membrane lipids [48]. Killing unfavorable bacteria required high pulsed voltages (25 kV cm^{-1} and 40 - 100 μs pulse duration) and this is related to neither interaction with the products of electrolysis nor with temperature changes but rather a direct effect of the current on the cells [49]. The effect of direct current application on different strains of bacteria in liquid and slurries has been investigated [49]-[51]. Therefore, electrokinetics has the potential to be that tool. There is a need for further research to be conducted to develop this area.

Recent advancement in biotechnology and molecular tools has enhanced the production and recovery of enzymes. Many authors have suggested the use of enzyme (Biocatalysis) in bioremediation instead of microorganism [52] [53]. The use of enzyme in bioaugmentation can result in avoiding the competition between indigenous bacteria and the new strains. The advantages of using enzymes in bioaugmentation are enzymes can simplify the process (they do not generate by-products), it is easier to work with enzymes than with the whole microorganism, enzyme capabilities can be improved at the production stage. However, the cost of enzyme production is high. Also there is an issue about shelf life and stability of the enzymes. The use of enzymes has not been investigated in EK bioremediation [53]. Enzyme delivery via electrokinetics transport mechanisms is a new research area and there is a need to investigate the efficiency of electrokinetic in delivery of enzymes to contaminated zones.

4. Electrokinetic Processes

Phenomena associated with the application of electrokinetics (electrolysis reactions, electromigration, electroosmotic flow, electrophoresis) can alter the physiochemical properties of the soil matrix and pore fluid [6] [54]. These changes, including the development of pH and voltage gradients, formation of zones with different current density, variation of electric current and voltage gradient, and an increase in temperature of the soil, can play a significant role in the outcome of an electrokinetic bioremediation processes. Electroosmotic flow can enhance bioavailability by stimulating desorption of contaminants from the soil.

4.1. PH Gradient

The Electrolysis reactions of water occur at the electrodes in an electrokinetic process and result in oxidation-reduction reactions. Oxidation takes place at the anode, which generates hydrogen ions (acid front H^+) and liberates oxygen gas. On the other hand, reduction occurs at the cathode, which produces hydroxyl ions (base front

OH^-) and disperses hydrogen gas.

Oxidation reaction at the anode:

$$H_2O - 4e^- \rightarrow O_2 + 4H^+ \quad [20]$$

Reduction reaction at the cathode:

$$H_2O + 4e^+ \rightarrow H_2 + OH^- \quad [55]$$

The acid front (*i.e.* H^+) moves towards the cathode by electroosmotic flow, diffusion, and electromigration and lowers the pH of the soil along its path. The hydroxide ions the form the base front travel towards the anode by electromigration and diffusion and elevate the pH of the soil in the vicinity of the cathode. The drastic change in soil pH (acidic near the anode and alkaline near the cathode) plays a very important role in the outcome of the removal of heavy metals and other contaminants from soil by EK remediation and in the degradation of contaminants by an EK bioremediation process. Most of the heavy metals are soluble at a pH less than 7 and precipitate at a pH higher than 7. Typically, the soil pH in EK remediation near the anode is in the range 2 - 3.5 and near the cathode between 8 - 11 [56]. For example, copper and cobalt are found in solutions in the pH range between 4 and 6 while they precipitate (for example, as insoluble hydroxides) at pH higher than 7. Thus, the decrease in soil pH (near the anode) is favorable for heavy metal dissolution and hence removal. However, the increase in soil pH can cause precipitation of heavy metals and render the technique ineffective in removing contaminants in the vicinity of the cathode. On the other hand, in EK bioremediation there is an optimum pH at which the capability of bacteria in degradation of a particular contaminant is optimum. Most bacteria can live in a pH range between 6 and 8. Special strains of bacteria can tolerate extreme pH values (<2 or >10). Bacteria can adapt the cytoplasm pH to the surrounding environment by controlling the exchange of H^+ (internal proton concentration) through the cell wall. However, the abrupt change in pH gradient across cell membrane has an adverse effect on growth and metabolism of bacteria [6] [12] [13].

To address the challenges caused by the pH gradient, researchers have previously implemented conventional and innovative techniques to control pH during electrokinetic remediation. The conventional techniques include the use of an ion selective membrane such as cation-exchange membrane, which prevents the transport of the hydroxide ions from the cathode to the soil as shown in **Figure 1** [57], continuous changing/removing of the solution in the electrode compartments [58], addition of chemical conditioning agents such as ethylenediaminetetraacetic (EDTA) [59] [60], acetic acid [55], and nitric acid [61]. Innovative techniques on the other hand include a stepwise moving anode [62] [63], polarity exchange [12] [64], circulation of an electrolyte (anolyte and catholyte) solution in the electrode compartments (see **Figure 2**) [35] [43] [65], and the two anodes technique (TAT) (see **Figure 3**) which has investigated the control of the advancement of the acid and the base fronts. The soil type (mostly buffer capacity) and the presence of anions which contribute to the buffer capacity (besides carbonates, hydrocarbonates and hydroxides): borates, phosphates, silicates and organic acids anions influence the pH changes and should be taken into account when choosing the right pH-regulation technique [63].

Many researchers have investigated the effect of pH on electrokinetic bioremediation using conventional methods. For instance, the use of electrokinetic bioremediationto mitigate creosote-polluted clay soil was investigated [58]. In this study, the soil pH was kept relatively unchanged by continuously changing/removing the solution in the electrode compartments. This technique not only involves additional cost but might not be suitable for field applications. Moreover, the practice of replacing the electrolyte solution produces a polluted solution

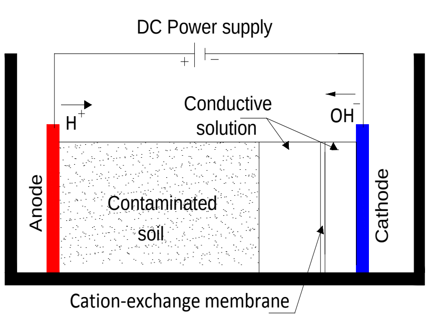

Figure 1. EK remediation configuration with ion selective membrane (after Li and Neretniek, 1998).

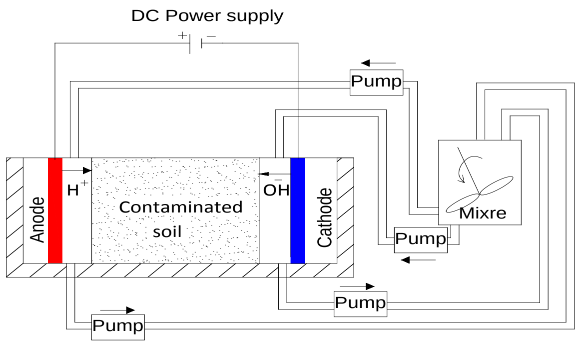

Figure 2. EK remediation cell with electrolyte solution technique (after Wu *et al*. 2012).

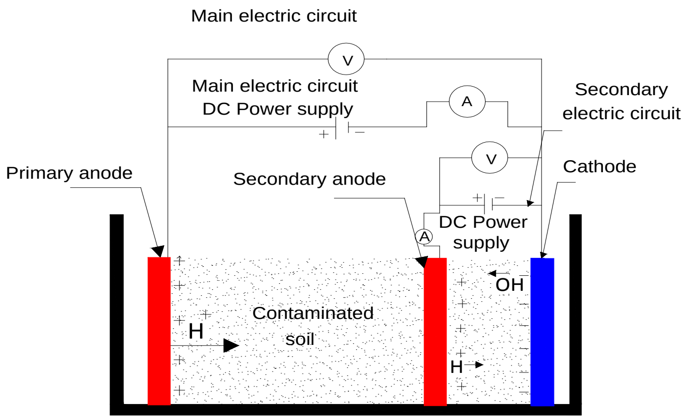

Figure 3. Two anode technique (TAT) (after Hassan and Mohamedelhassan 2011).

that requires treatment before being released into the environment. The addition of a chemical conditioning agent is not favorable because it generates by-products that may be toxic and harmful. Furthermore, the use of acid to control pH can acidify the contaminated soil, which is very difficult (if not impossible) to restore to its previous condition [66] [67].

The innovative techniques that have been proposed to overcome the negative impact of the pH gradient are either costly or not suitable for EK bioremediation in field applications. The step moving anode involves extra field work, as the anode should be advanced (relocated several times) towards the cathode during the process. Also, the technique is only suitable for EK remediation of heavy metals because the advancement of the anode creates an acidic environment all through the contaminated soil (pH ≤ 5) which results in desorption of heavy metals from soil but is not recommended for bioremediation. Likewise, the two anode technique is not suitable for EK bioremediation. In EK bioremediation, the low pH has a detrimental effect on bacteria. The polarity exchange technique relies mainly on the preciseness of pH measurement during the treatment and the current intensity. The soil pH and water content of phenol-contaminated soil were controlled using the polarity reversal technique [12]. This technique can be suitable for EK bioremediation, however, continuous pH monitoring is required which is challenging and increases the overall cost of the process. Kim and Han [32] implemented the circulation of electrolyte solution as shown in **Figure 2**. This technique can be effective and suitable for EK bioremediation; however, the field application can be costly because of the need for continuous pumping operation. Therefore, the circulation of electrolyte solution (anolyte and catholyte) in the electrode compartments is a troublesome technique for field application.

In electrokinetic (EK) bioremediation applications, the control of soil pH is crucial for successful treatment. Methods presented in recent literature show great advancements in the effort to control pH during EK application. However, more research is needed to improve existing methods and to develop new or innovative techniques to control the pH during EK bioremediation application, in particular. Currently, the authors are investigating a new technique to stabilize pH and to distribute nutrients uniformly during electrokinetic bioremediation (**Figure 4**). The new technique uses an anode and a cathode at the same water compartment. The hypothesis is that the coexistence of an anode and a cathode in the same water compartment will result in the hydrogen ions generated at the anode neutralizing the hydroxyl ions produced at the cathode and thereby forming water. In accordance with Equations (1) and (2), the proposed novel configuration is assumed to generate equivalent numbers of hydrogen ions and hydroxide ions with all the ions reacting to form water. Therefore, the new technique overcomes the shortcomings of other pH stabilization techniques by stabilizing the pH without the need for pumping or amendments while maintaining electroosmotic and electromigration movement in one direction.

4.2. Electric Current Density and Voltage Gradient

In general, the application of electric current through specific medium can cause direct and/or indirect effects on existing microorganisms. An example of direct effect is a rupture in the cell membrane due to a voltage gradient greater than 0.4 V across the cell wall [51] [68]. Indirect effects include the generation of by-products that are harmful to the microorganisms such as corrosion products introduced by metallic electrodes, which dissolve due to electrolysis reactions [69]. Much of the research has been conducted to investigate the effect of electric current on viability of bacteria for disinfection purposes and in the food industry [70] [71]. For instance, the use of high pulse DC current to kill yeast and bacteria investigated [49]. It was concluded that DC current, and not temperature or products of electrolysis, caused the death/inactivation of living organisms. Over the last decade, researchers have investigated the influence of electric current on electrokinetic bioremediation treatment. **Table 2** summarizes the results of studies that investigated the effects of electrical current on survival/transport of microorganisms during electrokinetic remediation. The effect of fixed applied electric current on different intensities of bacteria suspended on liquid and soil slurry was investigated [50]. It was found that electric current had a detrimental effect on low cell densities, however, high cell densities survived despite the applied electric field intensities and the controlled current environment [72]. The use of electrokinetic bioremediation to remove pentadecane from a kaolinite soil showed that the optimum pollutant removal was achieved using an intermediate electric current density of 0.63 mA/cm^2 compared with the higher and lower current densities of 3.13 and 1.88 mA/cm^2, respectively [72]. Another study showed that using optimum electric field in electrokinetic bioremediation not only removes pollutants but also retains the most microorganisms [73]. The results showed 37% of total petroleum hydrocarbons were removed from the area near the anode with an optimum electric field of 2 V/cm. In a recent study, it was observed that microorganisms were capable of degrading organic matter after being transported under an electric field [42]. Very few studies investigated the effect of the electrode materials on the electrokinetic bioremediation. For instance, the results of an experimental study [74] showed that the electrochemical reactions between the electrode material and the soil medium products significantly affected the activities of the microbial community. Although that study highlighted the importance of the electrode material in the process, the possible chemical reactions and the by-products were not detected. That study also concluded that the combined effect of applied current intensity and duration is the crucial factor affecting living organisms rather than the current intensity alone. The effect of electrode materials in EK applications using different materials for anode and cathode including steel, copper, and carbon with different combination (anode-cathode) was

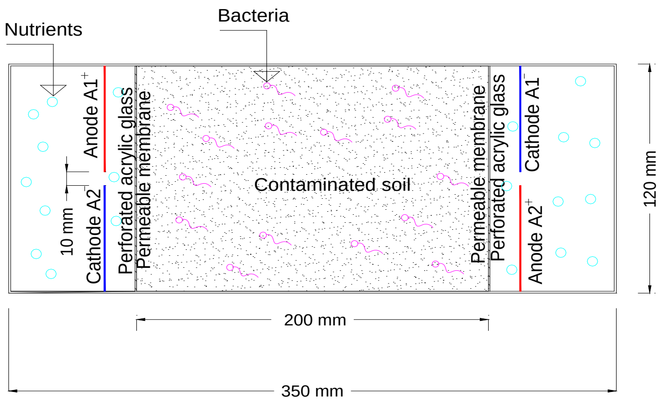

Figure 4. Anode cathode in the same compartment (ACC) (unpublished).

Table 2. Effect of electrical current.

Medium	Current intensity or voltage gradient used	Highlights/main outcome	Reference
Liquid and Soil slurry (silt)	20 mA/cm^2	High cell density survive despite to the applied current	[50]
Soil (kaolinite)	0.31, 0.63, 1.88, 3.13 mA/cm^2	Optimum current 0.63 mA/cm^2	[32]
Liquid	0.04, 4, 8, 12, 14 mA/cm^2	Optimum electric field density 100 kJ/L	[74]
Liquid	10.2 mA/cm^2	No effect on cell activity	[76]
Glass beads	1.8 mA/cm^2	Low level DC has no effect of cell viability	[77]
Soil (clay and silt)	0.314 mA/cm^2	pH changes near the anode is major factor affecting the microbial communities	[78]
Soil	1.0 mA/cm^2	If pH is controlled no negative effect from applied electric field on indigenes bacteria	[79]
hide-soak liquors	2 A (data are not enough to determine the current intensity)	Deactivated bacteria	[80]
Activated sludge	0.5 - 1.5 mA/cm^2	pH or direct contact caused bacterial inhibition	[81]
Fine grained soil	2 V/cm	The population of bacteria increased near the cathode	[73]
Sandy loam	0.46 v/cm	Rate of transport is 0.11 cm/h Microorganisms are active after the transport process	[42] [82]
Tap water/Sludge	0.28 - 1.4 v/cm	Optimum voltage intensity is between 0.28 and 1.4 v/cm	[83]

investigate [75]. The results show that using a particular material as anode and another as cathode or vice versa can result in considerable differences in the electrode performance (efficiency). Therefore, there is a real need for research to be conducted to address the effect of electrode materials in EK bioremediation.

4.3. Temperature

Microorganisms can live in a wide range of temperatures (thermophile 45˚C to 120˚C, mesophile 20˚C to 45˚C, or psychrophile −20˚C to10˚C). However, microorganisms growth rate in general increases with increase in temperature and the microorganism optimum degradation capability occurs at temperature between 25˚C and 40˚C (Nyer, 2001; Van Hamme *et al*. 2003). Many researchers have reported an increase in temperature during electrokinetic processes. For example, a studyshowed that soil temperature increased between 5˚C and 20˚C with the maximum increase reported in the soil near the anode [84]. An increase in temperature up to 90^o C during field application of electrokinetic remediation of trichloroethylene was reported [85] [86]. Although, it is well documented that electrokinetic processes can generate heat and elevate the temperature of the soil in the treatment zone, however, the effect of temperature on electrokinetic bioremediation has not been fully investigated. Investigators tend to attribute the increase in biodegradation to nutrient delivery by EK [87]. Few reports have discussed the effect of temperature increase during EK bioremediation. For instance, the delivery of nutrients and oxygen to microorganisms in the soil was investigated [88]. It was suggested that the increase in temperature associated with the applied electric field has a positive impact on microbial activities. On the other hand, continuous application of electrokinetic remediation using high applied voltage for long duration can elevate the temperature inside the soil being treated. The high temperature has an adverse effect on the viability of microorganisms. Intermittent current was used to avoid the adverse impact of the high temperature [86]. The use of current intermittence not only controls the increase in temperature, but also enhances the outcome of the EK application [75].

4.4. Bioavailability

Bioavailability can be defined as the quantity of contaminants present in soil pore fluid at a given time with respect to metabolism of the soil biota [89]. Bioavailability is also sometimes defined as the fraction of contaminants that is ready to be consumed by microorganisms [90]. Upon the release of pollutants into a soil matrix, depending on environmental conditions, sorption of the pollutants by the soil matrix takes place. In the subsurface, the only mechanisms for desorption of contaminants from the soil matrix is back-diffusion. Therefore, desorption is the major factor controlling the bioavailability of contaminants. There are two schools of thought on bioavailability. Some researchers believe that bacteria can degrade a contaminant, even if it is attached to the soil matrix [91]. Other researchers consider desorption of contaminants from soil as a prerequisite (contaminants to be desorbed first from the soil matrix before bacteria can degrade it) [92] [93]. Electroosmotic flow creates flow within the double layer, therefore, can enhance desorption of contaminants [94]. Recently, the authors have investigated the effectiveness of the electroosmotic flow compared to hydraulic flow in stimulating desorption of organic contaminants [95]. It was found that the concentration of the contaminant in the effluent after desorption tests using electroosmotic flow is three to four times higher than the concentration in the hydraulic flow tests. Also, the power consumption during the hydraulic flow tests was three orders of magnitude higher than the power consumed during the electroosmotic flow tests.

5. Available Power Sources for Electrokinetics

Energy consumption is a major component of the total cost of electrokinetic remediation. High energy consumption increases the overall cost of the remediation process and can become a major obstacle restricting wide field applications of this technology. Although, the cost of energy represents 30% of the total cost of an electrokinetic remediation process [96], very few research projects have addressed the high energy cost [97]. Solar energy, a renewable energy source with no adverse environmental impact, is a novel power option for electrokinetics and can be economically viable, in particular, for remote sites without active power lines [97].

In the last decade solar energy has gained the attention of scientists and the general public, leading to a multitude of beneficial applications. According to Solar Buzz report [98], more than 70% of the photovoltaic (PV) resources have been installed in northern countries including Germany, Japan, USA, and Canada. More importantly, it has been observed that more electricity can be generated by PV panels during the wintertime because of sunlight reflection off snow, the albedo effect [99]. Although, solar cells can be an excellent candidate for power supply in electrokinetics, there is little or no published research that has investigated their use in electrokinetic bioremediation or the effect of the night-time off power cycle on the microorganisms. The use of solar cells as a source of power can reduce the electricity transmission expenses and eliminate power losses in the transmission lines. Furthermore, the power produced by solar cells is environmentally friendly. Also, solar panels produce DC electric field that is usable in electrokinetic applications without alteration (*i.e.* without the need for DC transformer). The expected reduction in solar cell prices as the technology continues to improve can significantly reduce the initial cost of a solar power system. The power generated by solar cell panel depends on the time of day and the weather conditions. This can cause fluctuations 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 of an electric field in electrokinetics results in ion orientation in the double layer against the electric current, which reduces the efficiency of the remediation process. Interruption of the electric field allows the restoration of original ions orientation, which can enhance the remediation process. It is suggested that the fluctuation of the power generated from solar panels during the day and the diminishment of electric field during the night would stimulate the remediation process. Many studies have proven that current intermittence is beneficial to the outcome of an electrokinetic process [75] [100]. In a previous study, the authors have used solar panels to generate power for the electrokinetic remediation of clay soil contaminated with copper [67] [101]. Three solar panels were used to generate 41, 27 and 13.5 V. The results showed that solar panels can be used successfully to produce enough power for electrokinetic remediation of heavy metal-contaminated soils. In recent work, the authors used solar panels to generate power for the electrokinetic bioremediation of 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 [102]. Also, it has been observed that, in some situations, the power generated by solar panels in the winter (snow covered ground) was higher than that produced in summer (albedo effect). Moreover, the intervals of zero voltage at night can decrease soil temperature in field applications, which is a benefit.

6. Field Applications

Electrokinetic (EK) remediation has been in use for a while to clean up sites contaminated with heavy metals as well as for ground improvement in laboratory and field scale. For instance, in previous studies the effectiveness of electrokinetic remediation field application in the removal of copper from a contaminated site with an average removal of 85% [103]. Many researchers have conducted laboratory testsusing electrokinetic bioremediation (**Table 3**). On the other hand, field applications are very limited. For example, Lasagna technique was usedto remove trichloroethylene (TCE) from a contaminated site, in Paducah, Kentucky [86]. At this site, EK was successful in cleaning up TCE form clay soil with removal between 95% - 99%. However, very few field studies have been conducted in electrokinetic bioremediation. The first field application of electrokinetic bioremediationwas conducted in Denmark in 2012 to degrade perchloroethylene (PCE) from clay soil. The dimensions of the site investigated were 1.8 × 3 × 2.7 to 7.2 m in width, length and depth, respectively. The results show that electrokinetics can be used successfully to deliver microorganisms capable of degrading perchloroethylene (pce).

7. Future Research

The Electrokinetic bioremediation can be an effective remediation technique suitable for field applications, provided that the process cost can be reduced and the pH gradient is controlled. High energy consumption increases the overall cost of the remediation process and may become a major factor restricting the field application of the technology. There are very few studies in the available literature that have investigated the cost of energy in electrokinetic bioremediation, which is a major contributor to the total cost of the process.

Research to date has shown a low to moderate percentage of contaminant removal using electrokinetic bioremediation. Future research should pay more attention to optimize the removal efficiency by electrokinetic bioremediation. In addition, current research tends to address the pH issue using two different approaches, either by using conventional techniques, in which chemical compounds are added to control the pH, or by conservative techniques, such as using a pump to circulate the anode and cathode compartment fluids in an attempt to neutralize the pH. Both techniques can result in a further increase in the overall cost of remediation. Moreover, the effect of the increase in temperature associated with electrokinetic bioremediation has not been fully investigated.

Advanced technologies, in particular, biotechnology and synthetic biology provide great opportunities for enhancing electrokinetic bioremediation with reduced cost. This may involve several research components including, prolonging the survival and function of the microbes in contaminated sites, identifying new bacterial strains with better performance in bioremediation, enhancing the metabolic ability of indigenous bacteria through microbial genetic engineering, exploring the rich microbial sources for powerful contaminant degrading enzymes using metagenomics, and designing smart engineering tools for efficient bioremediation. It is anticipated that, with intense research efforts, electrokinetic bioremediation would become a viable technology in the near future.

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Table 3. Type of contaminant.

Medium	Contaminant	Contaminant concentration	Highlights/main outcome	Reference
Fine soil (from a contaminated site)	Petroleum hydrocarbon	78,600 mg/kg	37% reduction	[73]
Sand	Diesel	6800 mg/kg	60% reduction	[72]
Clayey soil	Phenanthrene	200 mg/kg	65% removal	[34]
Coarse sand/sand	Creosote	50, 200, 500, 900, 6800 mg/kg	50%, 68%, 80% reduction	[88]
Sandy loam	Phenol	180 mg/kg	58% reduction	[104]
Clay	Creosote	1300 mg/kg	35% reduction	[58]
Kaolinite	Pentadecane	1000, 5000, 10,000, 20,000 mg/kg		[32]
Sandy loam	Phenol	200 mg/kg	49%, 60%, 67% reduction	[12]

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