ELECTROKINETIC BIOREMEDIATION TECHNIQUE ON CONTAMINATED LANDFILL SOIL BY *PSEUDOMONAS PUTIDA*

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This thesis is dedicated to my beloved husband and parents, siblings and friends.

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ABSTRACT

Currently, many researchers are finding solutions to remediate heavy metal contamination in soil. The Electrokinetic Remediation (EK-R) treatment is one of the methods that can be used for the removal of heavy metals. By combining with other techniques such as Bioremediation would likely increase the effectiveness of the EK-R technique. The objective of this research is to investigate the ability of Electrokinetic Bioremediation (EK-Bio) technique to remove Hg, Cu, Zn, As, Pb, nitrate and sulphate from Bukit Bakri landfill soil at Muar, Johor. *Pseudomonas putida* (*P.putida*) was used in this research because it can survive easily under various conditions. *P.putida* was used in the EK-Bio technique as an agent to improve the performance of Hg, Cu, Zn, As, Pb, nitrate and sulphate removal from contaminated landfill soil. Research were conducted for durations of 1, 2, 3, 5, 7 and 14 days under low voltage (1 V/cm) and ambient temperature. The results showed that the EK-Bio treatment can remove up to 61 %, 95 %, 69 %, 71 % and 51 % of Hg, Cu, Zn, As and Pb respectively at distance of 3 cm from the anode. Meanwhile, 99.6 % and 98.5 % of nitrate and sulphate were removed respectively at a distance 15 cm from anode. Besides that, the enumeration of *P.putida* for all the EK-Bio treatments increased with the duration of treatment. Thus, this research showed that the EK-Bio treatment was able to increase the removal of Hg, Cu, Zn, As, Pb, nitrate and sulphate in contaminated landfill soil. It can be concluded that the EK-Bio treatment using *P.putida* potentially can be used to treat the contaminated soil.

ABSTRAK

Pada masa kini, ramai penyelidik telah membuat kajian untuk menyelesaikan masalah tanah yang tercemar dengan logam berat. Rawatan Elektrokinetik (EK-R) adalah satu kaedah yang telah dilaksanakan untuk menyingkir logam berat. Dengan menggabungkan teknik ini bersama dengan teknik lain seperti Bioremediasi mungkin dapat meningkatkan keberkesanan teknik EK-R. Objektif utama kajian ini adalah untuk menyiasat kebolehan teknik Bioremediasi Elektrokinetik (EK-Bio) untuk menyingkirkan Hg, Cu, Zn, As, Pb, nitrat dan sulfat daripada tanah pelupusan sampah Bukit Bakri di Muar, Johor. *Pseudomonas putida* (*P.putida*) telah dipilih dalam kajian ini kerana ia mudah untuk hidup dalam pelbagai keadaan. *P.putida* digunakan dalam teknik EK-Bio sebagai agen untuk meningkatkan prestasi penyingkiran Hg, Cu, Zn, As, Pb, nitrat dan sulfat di dalam tanah pelupusan sampah yang tercemar. Kajian ini telah dijalankan selama 1, 2, 3, 5, 7 dan 14 hari dengan voltan yang rendah (1 V/cm) pada suhu ambien. Keputusan menunjukkan rawatan EK-Bio telah menyingkirkan 61 %, 95 %, 69 %, 71 % dan 51 % Hg, Cu, Zn, As dan Pb masing-masing pada jarak 3 cm daripada anod. Manakala, 99.6 % dan 98.5 % nitrat dan sulfat telah disingkirkan pada jarak 15 cm daripada anod. Selain itu, perhitungan jumlah *P.putida* dalam semua rawatan EK-Bio semakin meningkat berdasarkan tempoh rawatan. Oleh itu, kajian ini menunjukkan rawatan EK-Bio dapat meningkatkan penyingkiran Hg, Cu, Zn, As, Pb, nitrat dan sulfat dalam tanah pelupusan sampah yang tercemar. Ia dapat disimpulkan bahawa rawatan EK-Bio dengan menggunakan *P.putida* berpotensi untuk merawat tanah yang tercemar.

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LIST OF ABBREVIATIONS AND SYMBOLS

^{o}C	Degree celcius
µM	Micromole
µm	Micrometer
As	Arsenic
B	Boron
BS	British standard
Cd	Cadmium
Cfu	Colony forming unit
cm^3	Cubic centimeter
Cu	Copper
Cr	Chromium
d	Day
DW	Distilled water
e^-	Electron ion
EPA	Environmental Protection Agency
FAST	Faculty of Science and Technology
Fe	Iron
FKAAB	Faculty of Civil Engineering and Built Environment
FRTR	Federal Remediation Technology Roundtable
FTK	Faculty of Engineering Technology
g	Gram
H^+	Hydrogen ion
H_2	Hydrogen

Hg	Mercury
HNO_3	Nitric acid
H_2O	Water
ICRCL	Interdepartmental Committee on the Redevelopment of Contaminated Land
kg	Kilogram
l	Litre
M	Mole
Mn	Manganese
mg	Miligram
ml	Mililitres
mm	Milimeters
N	Nitrogen
Ni	Nickel
NO_3^-	Nitrate
O_2	Oxygen
OH^-	Hydroxyl ion
P	Phosphorous
PAH	Polycyclic aromatic hydrocarbon
Pb	Lead
PHE	Phenylalanine
ppm	Part per millions
ppb	Part per billions
rpm	Revolutions per minute
Se	Selenium
SO_3^-	Sulphate
TPH	Total petroleum hydrocarbons
UTHM	Universiti Tun Hussein Onn Malaysia
US EPA	United States Environmental Protection Agency
V	Voltage
V/cm	Voltage per centimeter
Zn	Zinc

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CHAPTER 1

INTRODUCTION

1.1 Background of research

Malaysia is currently facing many problems that affect the environment, especially soil pollution. Contaminated soil refers to the presence of toxic chemicals in high concentrations in soil that may affect human health and the ecosystem. The main causes of soil pollution are human activities or nature. Human activities such as industrial activities and agricultural activities often produce solid waste. Industrial activity and manufacturing processes have become the largest contributor to soil pollution. During these processes, chemicals such as petroleum, polynuclear aromatic hydrocarbons (PAH), solvents, lead, pesticides and other heavy metals are used. In addition, it has been reported that the latest total population in Malaysia has increased from 8.2 million in 1960 to around 30.4 million in 2014 (Department of Statistics Malaysia, 2010). Both the increase in population and rapid economic growth in Malaysia have caused the excessive production of solid waste. The methods for waste disposal are based on the waste composition, operational budget and availability of technology. Responsible agencies must find a solution before this problem gets worse. The co-disposal for municipal waste that contains hazardous waste, incompetent utilisation of disposal areas, lack of environmental awareness and impoverished documentation are also among the contributing factors of soil pollution (Ismail & Manaf, 2013).

According to previous researchers, there are several ways to treat soil contamination such as bioremediation, phytoremediation, electrokinetic remediation, electrokinetic stabilisation, electrokinetic biological remediation (electrokinetic bioremediation), electrokinetic soil flushing, chemical precipitation, ion exchange and so on (Cameselle, Chirakkara & Reddy, 2013; Akhtar, Chali & Azam, 2013; Ramadan, Effendi & Helmy, 2018). Remediation is a method for saving the environment from contamination meanwhile bioremediation is a common technology which uses microorganisms to treat biodegradable contaminants. Bioremediation is a process that makes use of microorganism activity to remove organic contaminants or inorganic compounds (Gill *et al*., 2014). Bioremediation is categorised as a remediation technique for maintaining the cleanliness of the environment. This technique is based on microbial activity in soil which will degrade or absorb the contaminants from the environment due to their metabolic ability by aerobic or anaerobic microorganisms (Perpetuo, Souza & Nascimento, 2000). Normally, the bioremediation process uses microorganisms such as bacteria, yeast, fungi or plants as a biodegradation or biotransformation agent. This remediation can be used for contaminated groundwater, soils, lagoons and sludge (Boopathy, 2000). The advantages of bioremediation are its cost-effectiveness and eco-friendliness (Schmidt, Barbosa & Almeida, 2007). The Environment Protection Agency Guidelines (2005) stated that bioremediation can remove a variety of organic chemicals, heavy metals (Guo *et al*., 2010; Nanda *et al*., 2011; Milton & Reetha, 2012; Akhtar *et al*., 2013), hydrocarbons (Margesin, Zimmerbauer & Schinner, 2000), diesel and biodiesels (Sunar *et al.*, 2012). However, the microbes cannot degrade heavy metals directly. Instead, they can transform the pollutants into less toxic products or immobilise pollutants by changing the valence of metals (Akhtar *et al*., 2013).

To improve the remediation of contaminated soil, the electrokinetic technique was introduced to be used in combination with the bioremediation technique. Electrokinetic remediation (EK-R) is also known as electrokinetic soil processing, electrochemical decomposition, electromigration or electroreclamation. Electric current extracts certain elements such as heavy metals, radionuclides, organic compounds, or mixed inorganic species and organic waste from the soils and slurries (Acar *et al*., 1992; Azhar, 2012; Jamil *et al*. 2014; Rosestolato, Bagatin & Ferro, 2015) due to electroosmosis, electromigration

and electrophoresis which occur during electric current supply (Gill *et al*., 2014). When electric current passes through the soil, it is affected by ions and soil condition. Besides that, the transportation of ions through electromigration, electroosmosis and electrophoresis during EK-R is due to the pore fluid present in soil. At the anode, the soil becomes acidic due to the large amount of H^+ ions produced while soil at the cathode becomes alkaline due to the large amount of OH^- ions produced through the migration of that ions (Li *et al*., 2010). According to Kim *et al*. (2011), EK-R focuses on the removal of heavy metals in contaminated soil during the initial stage. Some researchers used EK-R for different types of contaminants to treat contaminated soils such as recalcitrant soil and hydrophobic organic soil (Cameselle *et al*., 2013). Reddy *et al*. (2003) found this method effective for removing heavy metals such as lead, copper, chromium, cadmium and nickel in various types of soil.

Based on the remediation performance of contaminated soil, electrokinetic bioremediation (EK-Bio) was selected as a hybrid technology to treat contaminated soil. According to previous researches, this combination technique was not used to treat inorganic pollutants but it was widely used to treat organic pollutants for example diesel (Li *et al*., 2010; Mena *et al*., 2015), phenantherene, polyaromatic hydrocarbon and total petroleum hydrocarbon (Acuña, Pucci, & Pucci 2012). Nevertheless, this research used the hybrid technology to treat contaminated landfill soil which contains heavy metals, nitrate and sulphate. This technique used low intensity direct current through a pair of inert electrodes inserted into the contaminated soil as well as the ability of microorganisms to remove and transform the contaminants. Heavy metal ions, nitrate and sulphate migrated to the opposite charge whereas the microorganisms used trace metals, nitrate and sulphate as a nutrient source (Duxbury, 2000).

In this research, *Pseudomonas putida* (*P.putida*) was the microorganism chosen to increase the removal of heavy metals. The selection of *P.putida* was based on its survivability in variable habitats such as in water, soil or plants (Lyczak, Cannon & Pier, 2000). The optimum temperature for the growth of *P.putida* is 35 ^oC (Kucerova, 2006) while the growth is reduced when the temperature is below 27 ^oC (Munna, Zeba & Noor, 2016). Besides, some strains of *P.putida* are not pathogenic to human health and the environment due to the lack of some genes including enzymes (Emparan, 2015). *P.putida*

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