BIOSURFACTANT ENHANCED BIOELECTROKINETIC REMEDIATION OF PETROCHEMICAL CONTAMINATED SOIL

BRIAN GIDUDU

A dissertation submitted in partial fulfilment of the requirements for the degree:

MASTER OF ENGINEERING IN ENVIRONMENTAL ENGINEERING

FACULTY OF ENGINEERING BUILT ENVIRONMENT AND INFORMATION TECHNOLOGY

UNIVERSITY OF PRETORIA

2019

ABSTRACT

Title: Biosurfactant Enhanced Bioelectrokinetic Remediation of Petrochemical Contaminated Soil

Author: Brian Gidudu

Supervisor: Professor Evans M.N. Chirwa

University: University of Pretoria

Faculty: Faculty of Engineering Built Environment and IT

Department: Chemical Engineering

Degree: Master of Engineering in Environmental Engineering

Soil pollution in recent years has emerged as an issue of great environmental concern. Contamination of soil by improper disposal or spillage of petrochemicals and products containing petroleum hydrocarbons is one of such pollution cases highly reported. To remediate petroleum contaminated soil, A DC powered electrokinetic reactor was used with biosurfactants as an enhancement for the remediation process. To begin with, studies were made under voltage variations of 10 V and 30 V with an electrode spacing of 185 mm. Biosurfactant with its producing microbes and biosurfactant free cells were introduced in the soil chamber after which the reactor was left to run for 10 days under the electric field. The technology was able to achieve the highest oil recovery of 75.15 % from the soil in 96 hours at 30 V. With other factors remaining constant, the reactor was also operated under a constant voltage of 30 V with configurations of fixed electrodes spacings of 335 mm, 260 mm,185 mm and continuous approaching electrodes at 335 mm, 260 mm and 185 mm. The current in the electrolyte was highest with the least electrode distance of 185 mm. The increase in current led to a direct proportional increase in the electroosmotic flow towards the cathode leading to increased coalescence of the oil from the soil as compared to the other electrode distances. The analysis of the results showed reduction in the total carbon content in the soil with viable oil recovery rates for all the

electrode distances with 185 mm being the most effective in both oil recovery and degradation. The reactor was further operated with amended biosurfactant concentrations of 28 g/L, 56 g/L and 84 g/L to enhance the recovery of oil from the soil and aid in biodegradation of the remaining oil by hydrocarbon degrading microbes. The highest oil recovery of 83.15 % was obtained with the biosurfactant concentration of 56 g/L showing that the hyper increase in concentration of the biosurfactants is not necessary to have an efficient process. In all experiments the microorganisms were able to survive under the electro-halo-thermal environment in the reactor and degraded the remaining hydrocarbons to acceptable amounts in the environment. The bacteria were however affected by the constantly changing pH in all experiments. The presence of biosurfactants was so significant in aiding oil recovery and increasing bioavailability of hydrocarbons to the microbes. Production of biosurfactants in the reactor followed up by kinetic suggestions of the processes in the bioelectrokinetic reactor should be studied in future.

Key words: Electroosmosis; Electrophoresis; bioavailability; biosurfactants; petrochemicals; oil recovery.

DECLARATION

I **Brian Gidudu** hereby declare that the work presented in this thesis for the award of Master of Engineering in Environmental Engineering is my own work and has not been previously submitted by me to this institution or any other institution.

……

Brian Gidudu

This …….. day of ……. 2019

ACKNOWLEDGEMENTS

Above all, I would like to thank God the almighty for the gift of life, good health, abundant blessings, guidance, and all sorts of providence for enabling to successfully accomplish this work.

Without the slightest of reproach, I would like to express my sincere gratitude and extreme appreciation to my supervisor Professor Evans M.N. Chirwa for allowing me to do this research under his invaluable custodianship, guidance, mentorship, and sponsorship to its end. Without his motivation, always positive attitude to research, sincerity, leadership, kindness, contriving imagination and vision this research would have only but disappeared in thin airs.

I am exceptionally grateful to my family Mr Fred Gidudu, Mrs. Jenipher Gidudu, Dr. Aaron Gidudu, and Eng. Henry Gidudu for their sacrifices, love, encouragement and prayers. I will indeed forever be indebted to you all.

I would also want to pass special appreciation to the Mastercard Foundation for offering me a scholarship to enable me to reach my academic goals. You are indeed the drivers of transformative change and leadership.

I also express my appreciation to Miss Alettee Devaga and Miss Elmarie Otto for the administrative support. Your assistance was central to the success of this research.

And lastly to all the lecturers, my research colleagues in the division and friends, your hospitality, friendship, support, and motivation is the reason this work was on the crest of a wave it started.

TABLE OF CONTENTS

ABSTRACT i
ACKNOWLEDGEMENTS iv
LIST OF ACRONYMS xiii
CHAPTER ONE 1
INTRODUCTION 1
1.1 Background 1
1.2 Problem Statement 5
1.3 Research Hypothesis 6
1.4 Project Objectives 6
1.4.1 Primary Objectives 6
1.4.2 Specific Objectives 6
CHAPTER TWO 7
LITERATURE REVIEW 7
2.1 Background 7
2.2 Contaminants found in oil 7
2.3 Effects of poor management of oil containing substances to the environment 9
2.4 Remediation of oil contaminated soil 12
2.5 Microbial Enhanced Oil Recovery (MEOR) 23
2.6 Surfactants 24
2.6.1 Types, characteristics and properties of surfactants 24
2.6.2 Biosurfactants and biosurfactant producing species 27
2.6.3 Screening of biosurfactant producing species 28
2.6.4 Identification of biosurfactant producing species 30
2.6.5 Biosurfactant production 33
2.6.6 Factors affecting biosurfactant production 34
2.6.7 Recovery, purification and characterisation of biosurfactants 36
2.6.8 Critical Micelle Concentration (*cmc*) 37
2.7 Bacteria and biosurfactants in bioremediation 38

2.7.1 The processes involved in the biodegradation of organic compounds 41
2.7.2 Factors affecting degradation of hydrocarbons 41
2.8 Electrokinetics.....45
2.8.1 The fundamental theory of electrokinetics 45
2.8.2 Electrodes and electrolytes 50
2.8.3 Electroosmosis and its effects to the remediation process 52
2.8.4 Electromigration and its effects to the remediation process.....54
2.8.5 Demulsification rate due to application of an electric current.....56
2.8.6 Current 57
2.8.7 PH distribution and its effects to the electrokinetic remediation process.....58
2.8.8 Effect of surfactants application in the electrokinetic cell.....59
2.8.9 Effect of the electrokinetic process on microorganisms.....64
2.8.10 Ex-situ application of the electrokinetic processing system.....67
CHAPTER THREE.....68
METHODOLOGY.....68
3.1 Materials.....68
3.1.1 Chemicals and Reagents 68
3.1.2 Petroleum contaminated soils 68
3.1.3 Microbial culture, media and growth conditions.....69
3.1.4 Screening for biosurfactant production.....69
3.1.5 Characterization and identification of microbial species 70
3.1.6 Biosurfactant production, recovery and purification.....71
3.1.7 Biosurfactant characterization 71
3.2 Scanning Electron Microscopy (SEM) for analysis of colloids in the anode compartment resulting from electrophoresis.....74
3.3 Electrokinetic set up.....75
3.4 Electrokinetic experiments.....75
3.4.1 Experiments to determine the effect of voltage.....75
3.4.2 Experiments to determine the effect of electrode distances 76
3.4.3 Experiments to determine the effect of biosurfactant concentrations77

3.5 Total Carbon analysis 77
CHAPTER FOUR 79
CULTURE ISOLATION AND BIOSURFACTANT PRODUCTION 79
4.1.2 Characterization of biosurfactants 80
4.2 Evaluation of the demulsification potential of the biosurfactants in O/W and W/O emulsions 83
CHAPTER FIVE 88
BIOELECTROKINETIC REMEDIATION WITH VARRIATION IN VOLTAGE 88
5.1 The effect of voltage on the electrokinetic remediation of petroleum contaminated soil 88
5.1.1 Oil Recovery 89
5.1.2 Current 92
5.1.3 In situ microbial growth 94
CHAPTER SIX 99
BIOELECTROKINETIC REMEDIATION WITH SPECIFIED ELECTRODE CONFIGURATIONS 99
6.1 Background 99
6.2 Variation in soil pH and microbial counts 100
6.3 Current and oil recovery 102
6.4 Electroosmotic Flow (EOF) 105
6.5 Hydrocarbon removal after electrokinetic remediation 107
CHAPTER SEVEN 109
BIOELECTROKINETIC REMEDIATION OF OIL CONTAMINATED SOIL WITH INCREASING BIOSURFACTANT CONCENTRATIONS 109
7.1 Background 109
7.2 Current 110
7.2 Electroosmotic Flow (EOF) and Electrophoresis 111
7.3 Zeta potential of the biosurfactants under different pH 113
7.4 Oil recovery 115
7.5 PH 118
7.6 Microbial counts 119

7.7 Hydrocarbon degradation.....121
CONCLUSIONS AND RECOMMENDATIONS.....124
APPENDICES.....127
Appendix 1: Bacterial counts in the electrode compartments form the beginning to the end of the experiment. 127

LIST OF FIGURES

Figure 3. 1. Schematic view of the electrokinetic reactor 75
Figure 4. 1. Phylogenetic tree based on the 16S rRNA genotype fingerprinting method with a scale bar corresponding to 0.020 estimated nucleotide distance per sequence position.....80
Figure 4. 2. Surface tension versus concentration of biosurfactants produced by *Pseudomonas strain* PA1 81
Figure 4. 3. Fourier-transform infrared spectra of the biosurfactant produced by the strain PA1.....82
Figure 4. 4. Analysis of Thin Layer Chromatography of the biosurfactant produced by the strain PA1 revealing a pink pigment after spraying with ninhydrin to produce an Rf value of 0.42 83
Figure 4. 5. Demulsification of O/W and W/O emulsions by the biosurfactant supernantant 85
Figure 4. 6. Demulsification effect of the biosurfactant supernatant on W/O emulsions of Toluene, Kerosene and n-Hexane 86
Figure 4. 7. Demulsification effect of the biosurfactant supernatant on O/W emulsions of Tween-Triton-Kerosene 87
Figure 5.1. Figure 5. 1. A-Set up at the beginning of the experiment; B-High electroosmotic flow from the anode to the cathode and Oil coalescence in the medium chamber after 24 hours; C-High oil stagnation at the cathode-medium interface; D-High oil flow to the anode compartment.....92
Figure 5. 2. Time course of current during the electrokinetic process 94
Figure 5. 3. Plate count agar plates showing the dominance of the Pseudomonas aeruginosa strain after 48 hours of running 96
Figure 5. 4. Average bacterial counts up to the end of the experiment. 96
Figure 5. 5. Average pH distribution up to the end of bio-electrokinetic treatment.....97

Figure 5. 6. Total Carbon remaining in the soil matrix after the experiments ...97
Figure 5. 7. Soil samples collected from the bioelectrokinetic reactor having different colours symbolizing the difference in carbon content left in the different sections of the medium after 240 hours; A-10 mm, B-30 mm, C-50 mm, D-70 mm, E-100 mm, F-130 mm, G-150.....98
Figure 6. 1. PH and bacterial count variations in different sections along the normalized distance from the cathode……...102
Figure 6. 2. Time course of current for 185, 260- and 335-mm electrode 104
Figure 6. 3. Oil recovery for different electrode spacing 104
Figure 6. 4. Time course of EOF of water 106
Figure 6. 5. Time course of EOF of water against the time course of current .107
Figure 6. 6. The average total carbon remaining in the soil after 240 hours of the experiment.....108
Figure 7. 1. Time Course of current and EOF flow in the reactor for 28 g/L, 56 g/L and 84 g/L biosurfactant amendments……112
Figure 7. 2. SEM micrograph of coagulated colloidal particles in the anode comparting after electrophoresis.....113
Figure 7. 3. Recovered oil, biosurfactants and a turbid electrolyte collected from the anode compartment after the experiment.....115
Figure 7. 4. Oil recovery and movement for different biosurfactant concentrations of 28 g/L, 56 g/L and 84 g/L after 240 hours. 117
Figure 7. 5. PH and bacterial count variations in different sections along the normalized distance from the cathode 121
Figure 7. 6. Time course of bacterial growth for 240 hours. 118
Figure 7. 7. Total carbon left in different sections of the reactor between 0-50 mm, 50-100mm and 100-160.5 mm from the cathode. 123

Figure A. 1. Bacteria counts determined in the cathode and anode compartments as a function of time.....127

LIST OF TABLES

Table 2. 1: Comparisons of some of the oil contaminated soil remediation methods as assessed and reported by other researchers (Hu et al., 2013, Lim et al., 2016, Islam, 2015).....19

Table 2. 2: The microorganisms responsible for specific types of biosurfactants as taken from Das and Chandran (2011), Uzoigwe et al. (2015) and Souza et al. (2014) 31

Table 2. 3: Bacteria used in petroleum hydrocarbon bioremediation processes 39

Table 2. 4: Application of surfactants in the removal of organic contaminants as adapted from Gomes et al. (2012).....62

Table 3. 1: Experimental conditions……..……76

Table 5. 1: Experimental conditions…….88

Table 5. 2: Volume of oil recovered in the soil compartment and that transferred to the electrode compartments due to electroosmotic flow in 96 hours 90

Table 7. 1: Zeta potential of the biosurfactants in pH at pH 13, 7 and 1……..……...114

LIST OF ACRONYMS

HHV	Higher Heating Value
INSPQ	Quebec National Public Health Institute
PAH	Polycyclic Aromatic Hydrocarbon
US EPA	United States Environmental Protection Agency
GC/MS	Gas Chromatography/ Mass Spectrometry
CMC	Critical Micelle Concentration
MSM	Mineral Salts Medium
CMD	Critical Micelle Dilution
HPLC	High Performance Liquid Chromatograph
TLC	Thin Layer Chromatography
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscope
PHC's	Petroleum hydrocarbons
NSO	Nitrogen Sulphur Oxygen
EOF	Electroosmotic Flow
W/O	Water-Oil emulsion
O/W	Oil-Water emulsion
EOR	Enhanced Oil Recovery
VOC's	Volatile Organic Compounds

LIST OF JOURNAL AND CONFERENCE ARTICLES

Journal Articles

1) Gidudu B., Chirwa E.M.N., 2019. Biosurfactant Facilitated Emulsification and Electro-Osmotic Recovery of Oil from Petrochemical Contaminated Soil. Chemical Engineering Transactions, 76.
2) Gidudu B., Chirwa E.M.N., 2019. Approaching Electrodes Configurations in Bio-electrokinetic Deoiling of Petrochemical Contaminated Soil. Chemical Engineering Transactions, 20.
3) Gidudu B., Mudenda E., Chirwa E.M.N., 2020. Biosurfactant Produced by Serrati Marcescens and its Application in Bioremediation Enhancement of Oil Sludge. Chemical Engineering Transactions, 79.
4) Gidudu B., Chirwa E.M.N. The combined effect of voltage, electrode spacing and application of biosurfactants on the bioelectrokinetic remediation of petroleum contaminated soil. Under review in Journal of cleaner Production since January 2020.
5) Chirwa E.M.N., Gidudu B., Tshilidzi B., Nembudani., Tichapondwa S.M., Mbedani P., Fayemiwo O.M. Biomineralisation of Low to High Molecular Weight PAHs in Oily Sludge and Soil Biopiles Using Novel Biofilm Forming Pseudomonad and Acinetobacter Species. Under review in Journal of cleaner Production since January 2020.

Conferences Articles

1) Gidudu B., Chirwa E.M.N., 2019. Biosurfactant Facilitated Emulsification and Electro-Osmotic Recovery of Oil from Petrochemical Contaminated Soil. *14th International Conference on Chemical and Process Engineering* 26-29 May, Bologna, Italy.
2) Gidudu B., Chirwa E.M.N., 2019. Approaching Electrodes Configurations in Bio-electrokinetic Deoiling of Petrochemical Contaminated Soil. *22nd Conference on Process Integration for Energy Saving and Pollution Reduction - PRES'19*, 20–23 October, Agios Nikolaos, Crete, Greece.

CHAPTER ONE

INTRODUCTION

1.1 Background

The emergence of the industrial revolution harboured the oil and gas industry as a diverse and vital part of the global economy even as of today. The expeditious growth of the petroleum industry and its products in the last three decades has resulted into a remarkable contribution to environmental pollution. The spillage or disposal of petrochemical products and their related wastes into the environment such as on land or in water may have prolonged effects after the contamination event with some ecosystems such as mangrove swamps and salt marshes experiencing the effects for decades after the event (Kingston, 2002). This is because the products and wastes generated from the production or use of such products are usually composed of petrochemical pollutants such as petroleum hydrocarbons (PHC's) which are composed of alkanes, cycloalkanes, benzene, toluene, xylenes, naphthalene, phenols, and various polycyclic aromatic hydrocarbons (Das and Chandran, 2011, Islam, 2015). The greatest concern regarding contamination by hydrocarbons lies in the mutagenic, carcinogenic and toxic characteristics of such contaminants (Caravaca and Roldán, 2003, Souza et al., 2014). Soil pollution has been part of the commonest pollution events in the recent years resulting from leaks, poor management, spillage, transportation and sometimes poor storage of these hydrocarbons (Mena et al., 2016). Souza et al. (2014) argue that when these hydrocarbons get into contact with soil, they may form four different relationships. These are formation of a vapor phase in which the hydrocarbons join the soil vapour and at times adsorb on the solid surfaces of the soil or dissolve in water. The other is the formation of a free liquid phase which can easily penetrate the soil into the lowest points reaching ground water. At times the hydrocarbons form a dissolved phase if the hydrocarbons are highly

hydrophobic forming a hydrocarbon layer on the surface of either the soil or soil water and a plume in ground water. And lastly the hydrocarbons can form a residual liquid phase where the hydrocarbons are highly viscous or are adsorbed to the surfaces of the soil particles.

The frequent occurrence of such pollution events has led to the advancement of the electrokinetic process as a promising technology that has the potential to remove organic pollutants from contaminated soil much as it requires more innovative improvements to be effectively applied extensively on a field scale (Popov et al., 2008). First operated and observed by Reuss in the application of direct current in a clay-water mixture in the 19th century followed by an over view kinetic proposition of the likely processes by Helmholtz and Smoluchowski, the electrokinetic remediation method (also known as electro-reclamation, electrokinetic soil processing and electrochemical decontamination) employs the use of a low-intensity direct current across an electrode pair on each side of a porous medium (Altin and Degirmenci, 2005, Xu et al., 2014). This causes electro-osmosis of the aqueous phase, migration of ions and electrophoresis of charged particles in the colloidal system to the respective electrodes depending on the charge of ions and particles (Xu et al., 2017). They are mainly five processes that make up the electrokinetics phenomenon and these include diffusion, electrolysis, electroosmosis, electrophoresis and electromigration (U.S.EPA, 1997, Altin and Degirmenci, 2005). The remediation of a solid matrix such as soil, slurries or sludge containing oil and water by the electrokinetic process to separate the three different phases (water, oil, and solids) involves different mechanisms. These include the movement of colloidal particles of solid phase towards the anode area as a result of electrophoresis due to the breakdown of colloidal aggregates in solid matrix under the influence of an electrical field and the movement of the separated liquid phase (water and oil) towards the cathode area as a result of electroosmosis (Ali and Alqam, 2000). Finely divided

solids in contact with oil and water can form solid-stabilized emulsions. These fine particles act as a barrier to prevent droplet coalescence because they adsorb at the droplet surface thus lowering the demulsification rate constant. (Elektorowicz et al., 2006). PHCs which are a major composition of petrochemical hydrocarbons are generally classified into aliphatics, aromatics, nitrogen sulphur oxygen (NSO) containing compounds, and asphaltenes (major preventers of coalescence) (Hu et al., 2013). Yeung and Gu (2011) argue that it is almost impossible to remediate contaminated soil to the satisfactory and admissible levels by applying electrokinetics alone citing that the application of the technology with other remediation technologies could greatly enhance the process. In such for electrokinetic process improvement, Electorowicz and Hatim (2000) argue that the electrokinetic treatment performance can be affected by several factors such as resistance, pH, emulsion stability, electrical potential, and spacing between electrodes and suggest that this process may be improved through the use of surfactants or reagents to increase contaminant removal rates at the electrodes.

It is considerably claimed that surfactants and biosurfactants can be applied to enhance phase separation for remediation of soil contaminated with organic compounds by enhancing the aqueous solubility and mobility of contaminants (Mulligan et al., 2001, Yeung and Gu, 2011, Mulligan, 2009, Reddy and Saichek, 2004). Composed of a hydrophobic head and a hydrophilic tail, surfactants have been described as amphiphilic compounds whose hydrophilic tail makes them dissolve in the water phase hence increasing solubility of PHCs, while the hydrophobic head makes them tend to gather at the interfaces to decrease the surface or interfacial tension and thus enhance the mobility of PHCs (Mulligan, 2009, Singh and Cameotra, 2004, Wang et al., 2007). Due to low solubility and hydrophobicity properties of organic contaminants, it is usually complex to remove them from a solid matrix unless a surfactant is applied to act as a flushing

agent (Wang et al., 2007). Through micellisation, surface tension reduction, increasing contaminant bioavailability to microorganisms, solubilisation and increased adsorption, surfactants increase the rate of contaminant removal by altering the surface properties of the matrix in the electrokinetic reactor leading to an enhanced electroosmotic flow (EOF) (Gomes et al., 2012, Batista et al., 2006). The use of synthetic surfactants is however associated with a range of problems such as environmental toxicity and resistance to biodegradation (Mulligan et al., 2001, Yeung and Gu, 2011). As compared to chemical surfactants, biosurfactants have received increasing attention since they exhibit greater environmental compatibility, more diversity, better surface activity, lower toxicity, higher demulsification ability, higher selectivity, and higher biodegradability (Bezza and Chirwa, 2017, Abalos et al., 2004, Wang et al., 2007). Biosurfactants can also be produced in situ using organic contaminants such as substrates without ignoring their tolerance for extreme variations in pH, temperature and ionic strength (Ron and Rosenberg, 2002, Mukherjee et al., 2006). In reports regarding the study of remediation techniques that can be coupled with electrochemical remediation to enhance the remediation process, very few studies have been carried out on the use of biosurfactants to enhance the electrochemical remediation process (Yeung and Gu, 2011). Furthermore, in the process of performing electrokinetic remediation, very few studies have also been done about the effect of the electrokinetic process on the survival, growth, movement and enzyme activity of the microorganisms in the system (Kim et al., 2010, Lear et al., 2007, Wang et al., 2009). In this study we investigated the synergetic application of biosurfactants and hydrocarbon degrading bacteria in the bioelectrokinetic remediation of petroleum contaminated soil.

The variation of pH, electroosmotic flow, electrophoresis, and current as result of different electrokinetic reactor configurations were analysed. The effect of voltage variation, electrode spacing and three different biosurfactant

concentrations on oil recovery, bacterial growth and hydrocarbon degradation were also assessed. The effect of pH on the polarity of biosurfactants and its transport mechanism was also evaluated. The study went on to further evaluate the destination of oil and biosurfactants due to electromigration, electroosmosis and electrophoresis.

1.2 Problem Statement

The contamination of soil with petroleum hydrocarbons forms a complex emulsion of water, heavy metals, hydrocarbons and solid particles. The components of oil such as high PAHs makes it hazardous. The spillage of these hydrocarbon compounds into soil causes ground water pollution due to leaching of pollutants such as phenols, heavy metals and presents an adverse impact on public health. There is existence of several remediation technologies but none of them is the best solution for all because depending on the characteristics of the contaminant and contaminated matrix, every technology may need to be configured in a specific way to attain effective contaminant removal or remediation processes (Vocciante et al., 2016). The choice of a proper solution for a specific waste stream is usually an issue of contention in which a conclusion must be met meticulously (Vocciante et al., 2016, Kim et al., 2014a). An environmentally sustainable method or clean technology that offers high contaminant removal efficiencies with a positive response to the current climate change problems and energy crises in terms of greenhouse gases emission reduction, land and water pollution prevention would be the most ideal in this era (Vocciante et al., 2016, Elektorowicz and Habibi, 2005, Kim et al., 2014a, Altin and Degirmenci, 2005). Electrokinetic remediation has the potential of reducing the Global Warming Potential (GWP) per m^3 treated by 30 % and that is besides the fact that it poses a negative impact 10 times lower than the conventional treatment methods such as landfilling if it is rightly configured (Vocciante et al., 2016, Elektorowicz and Habibi, 2005, Kim et al., 2014a).

1.3 Research Hypothesis

In this research it is hypothesized that the application of biosurfactants to the oil contaminated soil will enhance the solid-liquid phase separation leading to recovery of oil but also the degradation of organic compounds that will be left in the soil after the oil recovery process.

1.4 Project Objectives

1.4.1 Primary Objectives

The primary objective of this research is to examine the possibility of applying biosurfactants as demulsification enhancers for increasing the separation of solid-liquid phases of oil contaminated soil in an electrokinetic reactor.

1.4.2 Specific Objectives

- To design and construct an electrokinetic reactor that can be used for the research studies
- To study the effect of electrode distance, applied voltage and pH on the remediation process
- To optimize the operation of the reactor by developing optimum reactor configurations.
- To determine the effect of pH and applied voltage gradient and electrode distance on the survival of bacteria.
- To determine the effect of biosurfactant concentration on the effectiveness of the reactor.
- To determine the rate of oil recovery and hydrocarbon biodegradation under optimum bio-electrokinetic reactor configurations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

A vast amount of petroleum contaminants are introduced into the physical environment during exploration, production, transportation, and refining of crude oil in the petroleum industry. These petroleum contaminants vary depending on the source of the crude oil, the process of refining, and the purpose for which the final product is meant for, but these petrochemicals include a valuable number of hydrocarbons in the company of other contaminants such as heavy metals and water among others. Oil waste is one of such oil production by-products that ends up in the environment. For instance, the USA produces 30,000 tons of petroleum sludge annually while China produces 3 million tons with some reports showing 60 million tons of sludge production annually (Pazoki and Hasanidarabadi, 2017). Oil recovery leads to the reduction in residual mass for further treatment such as biodegradation of contaminated slurries, sludge or soil hence reducing environmental impact and making it the most appropriate method for the management of oil-contaminated mediums (Pazoki and Hasanidarabadi, 2017). It should be noted that their cases were literature is scarce due to insufficient studies made previously.

2.2 Contaminants found in oil

Depending on the source, the oil may be composed of asphaltenes, heavy metals, heavy hydrocarbons such as long-chain paraffins, rusts resulting from surface oxidation of transportation and the storage containments (Pazoki and Hasanidarabadi, 2017). Basing on the source and chemical composition, oil extracts can be classified as chemical/Inorganic wastes, spent catalysts, hydrocarbon wastes, contaminated soils, and solids, and aqueous waste (Islam, 2015). The combination of oil and soil creates a stable W/O emulsion of solids

(colloids), water and metals preventing coalescence of similar molecules of water and oil. These emulsions are a result of the activity of emulsifiers such as asphaltenes, resins, oil-soluble organic acids, fine solids, nitrogen-oxygen and sulphur which are major components of petroleum hydrocarbons (Hu et al., 2013, Tyagi et al., 2011). Hydrocarbon waste is generally composed of dissolved air floatation float, desalter bottoms separator sludge, tank bottoms, slop oil emission solids, and waste oils/solvents. Spent catalyst includes hydro processing catalyst, fluid cracking catalyst, and others spent inorganic clays. Chemical waste may include spent acids, spent caustic, and waste amines (Islam, 2015). Contaminated soils and solids may be composed of waste sulphur, waste coke/carbon/charcoal, heat exchanger bundle cleaning sludge and miscellaneous contaminated soil while aqueous wastes include oil-contaminated water, high/low pH water, biomass, and spent sulphide solutions (Islam, 2015). Petroleum hydrocarbons are generally categorized in four ways as saturates, aromatics, resins, and asphaltenes. Resins may contain sulfoxides, quinolines, carbazoles, pyridines, and amides while asphaltenes may also contain ketones, fatty acids, phenols, esters, and porphyrins (Leahy and Colwell, 1990, Islam, 2015, Das and Chandran, 2011). Asphaltenes are only a major component in crude oil but absent in refined products, the other major constituent of crude oil are alkanes while alkenes only appear in refined petroleum products (Tyagi et al., 2011). The quantity of aromatic hydrocarbons such polycyclic hydrocarbons (PAH's) and total petroleum hydrocarbons depends on the properties of the used crude oil and processing operations of the refinery (Kriipsalu et al., 2008). The total petroleum hydrocarbon (TPH) contents in oily extracts can range from 5 % to 86.2 % by mass, but more frequently in the range of 15–50 %, whereas the contents of water and solids can be in the range of 30–85 % and 5–46 %, respectively (Hu et al., 2013). The high content of asphaltenes, resins, paraffin's, and other high molecular weight components in the oil sludge gets it characterised by high

viscosity properties, elevated solids content, and high aggregative stability (Egazar'yants et al., 2015).

Waste oil is listed as code A4060 under Annex VIII, List A of the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal (UNEP, 2014). In the List of Wastes Decision (EWC), the European Commission considers all waste oils except edible oils as hazardous (UK Environmental Agency, 2007). Numerous studies have reported the presence of most of these hazardous pollutants in oil including the study on k0-INAA measurement of levels of toxic elements in oil sludge and their leachability from samples obtained from Sg Udang, Melaka refinery from Malaysia which led to conclusions that the mean mass fractions of As, Cr and Zn in the oil sludge ranged between 61.5–67.9 mg/kg, 79.3–93.0 mg/kg and 1,760–1,830 mg/kg respectively (Fadzil et al., 2010). The United States' Agency for Toxic Substances and Disease Registry ranks arsenic as number 1 in its 2015 Priority List of Hazardous Substances. In the list also includes chromium and zinc in the top 100 listed hazardous substances (ATSDR, 2015). The Characterization and treatment of sludge from the petroleum industry in Nigeria using standard methods for the examination of water and wastewater and standard methods for water and effluents analysis also showed that the mean value of total solids, total suspended solids, total volatile solids, dissolved oxygen, the biological oxygen demand, chemical oxygen, nitrogen, phosphorus, the total bacteria counts greatly exceeded the standards set by the World Health Organisation for portable water (Asia et al., 2006).

2.3 Effects of poor management of oil containing substances to the environment

Environmental pollution by oil containing substances may have prolonged effects after the contamination event with some ecosystems such as mangrove swamps and salt marshes experiencing the effects for decades after the event (Kingston,

2002). The greatest concern regarding contamination by hydrocarbons lies in the mutagenic, carcinogenic and toxic characteristics of such contaminants" (Caravaca and Roldán, 2003). Gopang et al. (2016) argue that the organic materials in the oil affect the structure, compressibility and shear strength properties of the soil. The extent of soil contamination depends on the hydrocarbon characteristics and the soil properties (Caravaca and Roldán, 2003). Oil contaminated soil is said to have phytotoxic effects to red corn and red beans (Al-Qahtani, 2011). To determine the effect of oil sludge on plant growth and soil properties Al-Qahtani (2011) carried out a pot experiment on Vinca rosea (*Catharanthus roseus)* and soil composition which led him to conclude that dry matter yield of Vinca rosea decreased significantly with the application of oil refinery sludge. In addition, soil salinity and sodicity showed increases with application of oil refinery sludge. Oil contaminated soils can reduce the usability of land besides remaining bound to soils for years (Kisic et al., 2009). Soil contamination by petroleum hydrocarbons may lead to increase in concentration of radionuclides soil especially surface soil which may end up in the food chain (INSPQ, 2015).

When plants get coated with oil as a result of uncontrolled disposal, they may experience temperature stress and attenuated transpiration as a result of blocked transpiration pathways and blocked stomata respectively (Pezeshki et al., 2000). It is recognised that lighter weight oils are the more immediately toxic to plants and other organisms than heavier oils (Pezeshki et al., 2000). Aromatics can kill plants at 1% concentration but are more toxic to animals at greater concentrations (Nelson-Smith, 1968). In another study to determine the effects of oil pollutants on coral reef community, Loya and Rinkevich (1980) reported detrimental effects of oil pollution on reef corals, such as the complete lack of colonization by hermatypic corals in reef areas chronically polluted by oil, decrease in colony viability, damage to the reproductive system of corals, lower life expectancy of

planulae and abnormal behavioural responses of planulae and corals. They went on to highlight the other negative impacts included lower growth rates, direct damage to tissues, thinning of cell layers and disruption of cell structures, damage to tactile stimuli and normal feeding mechanisms, excessive mucus secretion leading to enhanced bacterial growth and eventual general coral destruction.

Petroleum hydrocarbons are said to accumulate in the tissues of marine organisms such as mussels (Kingston, 2002). The PAH's found in oil are known to have sub lethal damages to fish eggs and larvae even at extremely low concentrations (Langangen et al., 2017). The sub lethal effects in this case may among others include reduced feeding and growth rates, morphological deformities and are likely to increase vulnerability to predators and starvation (Langangen et al., 2017).

The acute impacts of direct human contact to petroleum hydrocarbons through inhalation and skin contact may include nausea, vomiting, headaches, dizziness and irritation of skin, eyes and mucous membranes as well as respiratory problems (INSPQ, 2015). The presence of heavy metals in oil poses a risk of cancer, birth defects, physical retardation, skin lesions, kidney damage, learning and mental disabilities, liver and a great number of other infections (Singh and Cameotra, 2004).

After spillage or poor disposal of oil, the fate of hydrocarbon compounds may take different pathways to different environment physical components with the influence of prevailing weather conditions. Kingston (2002) claims that the oil that finds itself in surface water spreads over the surface as a slick in a few millimetres thick as the toxic volatile compounds evaporate to the atmosphere. By a process known as photolysis some of the components in the oil are then oxidized to acidic and phenolic compounds making them more toxic while relatively toxic compounds with low molecular weight dissolve in water. The greatest impact of the release of volatile hydrocarbons is that they increase the

amount of greenhouses emitted to the atmosphere thus contributing to the greenhouse effects such as global warming (Elektorowicz and Habibi, 2005). A light hydrocarbon greenhouse contributor such as methane has a heat trapping effect 12 times higher than that of carbon dioxide (Elektorowicz and Habibi, 2005).

2.4 Remediation of oil contaminated soil

Recovery of oil from solid matrices is considered as an environmentally friendly strategy for waste handling which presents a number of oil recovery methods such as freeze/thaw treatment, solvent extraction, centrifugation treatment, surfactant enhanced oil recovery (EOR), sludge pyrolysis, electrokinetic method, microwave irradiation, Ultrasonic irradiation and Froth flotation as shown in table 2.1 (Hu et al., 2013). Recycling is the most desirable environmental option for handling oily contaminated slurries and sludge since it enables the petroleum industry to reuse valuable oil for reprocessing, reformulating and energy recovery. Moreover, recycling of oily sludge can reduce the disposal volume of hazardous waste outside the industrial zone, prevent the extent of contamination and decrease the use of non-renewable energy resources" (Hu et al., 2013). The recovery process is however feasible if the contaminated matrix contains at least 10 % oil (Zheng et al., 2012). The advantages, limitations, efficiencies, by-products and costs have been discussed in the details below and Table 2.1 to show some of the comparisons of some of the oil contaminated soil remediation methods. The comparisons show that the choice of a proper solution for a specific waste stream is usually an issue of contention in which a conclusion must be met meticulously. The descriptions of the different remediation methods of oil contaminated soil are described below.

2.4.1 Solvent extraction

This method is used to produce constituents of oil, water, and solids by breaking down complex molecules of slurries or sludge by solvents (Islam, 2015). When the solvent is applied to the oily matrix it dissolves the oil fraction and leaves the insoluble impurities at the bottom of the tank. The oil is then separated from the solvent in a solvent distillation system. The solvent can be recycled to repeat the process after cooling its vapour. The solvent remaining is also removed for recycling while the impurities are sent for further treatment. This method is mainly affected by the temperature, solvent, pressure mixing, solvent to sludge ratio which calls for the usage of large volumes of organic solvents, high energy consumption for heat generation and mechanical mixing (Islam, 2015, Hu et al., 2013, Lim et al., 2016).

2.4.2 Centrifugation treatment

In centrifugation treatment, water, solids, semi solids and oil are separated by a strong centrifugal force generated by a high-speed rotating machinery. The method requires a reduction of energy consumption, highly enhanced centrifugation efficiency and pre-treatment of the oily medium to reduce its viscosity by heating, steam injection or by adding demulsifying agents, organic solvents and tension active chemicals (Hu et al., 2013). The by-products including PHC's, water, and sediments separated after the centrifugation process are then collected for further treatment. The process is however associated with noise during operation and high energy consumption. The need for pre-treatment and application of demulsifying and tension active agents all lead to high operational costs (Hu et al., 2013, Islam, 2015).

2.4.3 Surfactant enhanced oil recovery (EOR)

In surfactant enhanced oil recovery, surfactants which are surface-active compounds with a hydrophobic tail and a hydrophilic head are used to remove

hydrocarbons from solid matrices (Kwon and Lee, 2015). The hydrophobic tail has the ability to make a surfactant molecule dissolve in the water phase and increase the solubility of hydrocarbons while the hydrophilic head has the ability to gather at the interfaces to decrease the surface or interfacial tension and thus enhance the mobility of hydrocarbons (Hu et al., 2013, Kwon and Lee, 2015, Islam, 2015). Some of the chemical surfactants used include sodium dodecyl sulphate (SDS), Tween 80, Triton X-100, Afonic 1412-7 and Corexit 9527 (Hu et al., 2013). Chemical surfactants have however been associated with environmental toxicity and low biodegradability in numerous studies. (Mulligan, Yong and Gibbs, 2001).

2.4.5 Freeze/thaw treatment

Demulsification is the major process of separating W/O emulsions in this method done by freezing the water ahead of the oil leading to the expansion of the water before the oil starts freezing (Hu et al., 2013). The result is a total disarrangement of the emulsion constituents which leads to coalescence of the oil and water droplets. In thaw treatment, the oil-water mixture is delaminated into two bulk phases while the oil phase is coalescing because of interfacial tension driven by gravitational force. Here the oil phase freezes ahead of the water phase forming a solid capsule which then breaks due to volumetric expansion of forming solid water capsules further forming crevices that allow contact of water molecules leading to aggregation. This forms an unstable oil-water mixture that is delaminated into bulk phases by applying a gravitational force. The thawing process is mainly affected by thawing and freezing temperatures, water content, the aqueous phase salinity, treatment duration, solid content and the presence of surfactants in the emulsion (Hu et al., 2013). The process may be more feasible in cold areas where natural freezing can be utilised as the process can be costly due to high energy requirements and the process is also very slow.

2.4.6 Oil sludge pyrolysis

Pyrolysis involves the decomposition of organic compounds in an inert environment at extremely high temperatures (500–1000 ºC). The recovery of oil by pyrolysis is affected by factors such as the rate of heating, temperature, the characteristics of the oily solid matrix, and the chemicals added to enhance the process. This can be done a number of configurations including fluid bed, Ablative pyrolysis, and circulating fluid bed pyrolysis, and vacuum pyrolysis. The process requires complex processing equipment, dewatering of matrices with high water content and high energy required for endothermic reactions which leads to high operational costs. The process also produces liquid products with high PAHs concentrations that are known to be carcinogenic (Hu et al., 2013).

2.4.7 Microwave irradiation

Microwave energy is used to provide improved and efficient quick heating to penetrate the oily matrix through interaction of its molecules with the electromagnetic field (Lim et al., 2016). This is different from conventional heating methods which transfer heat to the material through conduction, radiation, and convection of heat from the surfaces of the hot material. The recovery is done at frequencies between 900 MHz to 2450 MHz in industrial applications. The heat aids in demulsifcation of the W/O emulsions by reducing the viscosity of the constituents and accelerating the settlement of aggregating droplets (Hu et al., 2013, Islam, 2015). The water in the solid matrix ends up as the inner phase with a higher dielectric loss with the ability to absorb more microwave energy than oil. The absorption of energy by water results in its volumetric expansion with reducing water-oil interfacial film facilitating the separation of oil and water. The rearrangement of the electrical charges surrounding water molecules as result of microwave irradiation can also lead to molecular rotation which leads to destruction of the electric double layers at the oil/water interface. This leads to reduction of zeta potential, water and oil

molecules become more mobile leading to increased collisions and coalescence of oil and water droplets (Hu et al., 2013). The microwave irradiation process is however affected by the process duration, pH, surfactants, salt, microwave power and solid matrix properties. The application of microwave irradiation to industrial scale is generally limited due to high operating costs and the specific equipment required (Hu et al., 2013, Lim et al., 2016).

2.4.8 Ultrasonic irradiation

The demulsification of W/O emulsions in ultrasonic irradiation method is obtained by exerting rarefactions and compressions generated by ultrasonic waves (Lim et al., 2016, Agarwal and Liu, 2015). Rarefaction cycle separates molecules from each other by applying a negative pressure while the compression cycle pushes the molecules together by applying positive pressure. The negative pressure results in the production and growth of microbubbles to very unstable dimensions and collapse in violent manner leading to the generation of shock waves leading to further instant generation of high temperature and pressure. The increase in temperature of the emulsion, in turn, decreases its viscosity, increases the mass of the continuous liquid phase thereby destabilizing the W/O emulsion into separated solid and liquid phases in high concentrations. (Kwon and Lee, 2015, Islam, 2015). With efficiency ranging between 55.6 % and 95 % depending on the intensity and sonication power, the water content in the emulsion, temperature and solid particle size, it is undeniable that this method has high oil recovery efficiencies but has not been widely used for field-scale purposes because of the high cost of equipment and its maintenance (Hu et al., 2013, Lim et al., 2016).

2.4.9 Froth floatation

To acquire levitation, air bubbles have to capture oil droplets and small solids in an aqueous slurry where it can be collected in a froth layer. This is attained by

mixing the oily matrix with water followed by injection of air to form air bubbles. This results in the thinning of the water film between air and oil bubbles which ruptures to cause the spread of oil in air bubbles. Oil droplets conglomerate with air bubbles forming the oil to rise to the top of the surface where it can be skimmed off and purified (Islam, 2015, Lim et al., 2016). Depending on a number of factors including pH, the matrix properties, salinity, temperature, surfactant presence, the flotation duration and the size of the air bubbles, oil recovery efficiency may range between 55 % and 86 % (Agarwal and Liu, 2015). The method requires pre-treatment of the oily matrix to reduce its viscosity and further treatment and purification of the solid remains and recovered oil is required due to high moisture (Hu et al., 2013, Lim et al., 2016, Agarwal and Liu, 2015).

2.4.10 Incineration

The oil contaminants in soil are removed by combusting the soil in the presence of auxiliary and excess duels (Lim et al., 2016). Rotary kilns are the commonest with combustion temperatures in the range of 980-1200 ^{o}C. Other incinerators such as the fluidized incinerator combust at temperatures ranging from 730–760 ^{o}C. The fluidized incinerator is classified as being efficient due to its high mixing efficiency, low emission of pollutants, and efficient combustion abilities (Hu et al., 2013, Islam, 2015). Incineration reduces the volume of the contaminated soil which can then be used for other purposes but requires pre-treatment of the soil with high moisture content to reduce the consumption of energy during the remediation process. The process also leads to fugitive emission of pollutants into the environment which may also require special handling equipment hence the need for high capital investment and operating costs (Hu et al., 2013).

2.4.11 Bioremediation

Microorganisms are stimulated to grow by altering the environmental conditions of the soil so they can degrade specific pollutants (Lim et al., 2016). Bioventilation, biostimulation and bioaugmentation are generally the three mechanisms used in bioremediation (Lim et al., 2016). Bioaugmentation involves the introduction of genetically engineered bacteria in the contaminated soil to enhance the degradation process while Biostimulation involves the adjustment of environmental conditions of soil in which the bacteria have to grow by limiting or injecting nutrients to stimulate the growth of microorganisms and increase the biodegradation process. On the other hand, Bioventilation involves the injection of oxygen into the voids of soil to enhance growth of the microbes (Lim et al., 2016, Wu et al., 2016). The rates of uptake and mineralization of many organic compounds by microbial populations in the aquatic environment are proportional to the concentration of the compound, generally conforming to Michaelis-Menten kinetics (Leahy and Colwell, 1990). Hydrocarbons are susceptible to microbial degradation in different ways as follows in decreasing susceptibility: n-alkanes >branched alkanes > low-molecular-weight aromatics > cyclic alkanes (Leahy and Colwell, 1990). Degradation is usually highest in saturates, followed by light aromatics, then high molecular aromatics and polar compounds exhibit very low degradation rates (Wu et al., 2016, Leahy and Colwell, 1990).

Table 2. 1: Comparisons of some of the oil contaminated soil remediation methods as assessed and reported by other researchers (Hu et al., 2013, Lim et al., 2016, Islam, 2015)

Remediation methods	Application scale/status	Efficiency	Cost	By-products	Advantages	Limitations
Centrifugation	F			Unrecoverable sludge slurry and wastewater.	Fast, efficient and easy to apply, does not need chemical addition.	High consumption of energy, noise generation, needs pre-treatment to reduce viscosity, unable to treat heavy metals, requires high capital investments and maintenance.
Freeze/Thaw	L		----	Unrecoverable sludge slurry and wastewater.	It is suitable for cold regions, easy to apply, and has short treatment duration.	High operation costs due to need for freezing energy needs, Lower

						efficiency, unable to treat heavy metals.
Pyrolysis	F			Chars and VOCs.	Has large treatment capacity, fast and efficient, recovered oil can be upgraded.	High consumption of energy, high capital investments and maintenance, not suitable for oily sludge with high moisture content.
Solvent extraction	F			Unrecoverable sludge slurry and VOCs	Fast and efficient, easy to apply.	Unable to treat heavy metals, large amount of organic solvents is used, not environmentally sound and high cost of operation.
Surfactant (EOR)	F			Unrecoverable sludge slurry and wastewater	Fast and efficient, easy to apply, limited	Chemicals surfactants are toxic, high cost, need for removal of

					effect on heavy metals treatment.	surfactants from recovered oil.
Froth Flotation	L		-----	Generation of large amounts of wastewater	Low energy requirements, Easy to apply.	Uses large amounts of water, relatively low efficiency, unable to treat heavy metals, Unsuitable for treating oily sludge with high viscosity.
Ultrasonic irradiation	L		-----	Unrecoverable sludge slurry and wastewater	Fast and efficient, no need of chemical addition	High equipment cost, small treatment capacity, unable to treat heavy metals.
Electrokinetic	F		-----	Unrecoverable and sludge slurry	Fast and efficient, limited effect on heavy metals treatment, no need of chemical addition.	It's not easy to apply and has low treatment capacity.

Microwave irradiation	F		------	Wastewater, VOCs and unrecoverable solids	no need of adding chemicals, very fast and also efficient.	High energy consumption, high capital investment and operating cost, needs special designed equipment and unable to treat heavy metals.

Application status	L-laboratory scale		F-Field scale	
Efficiency (%)	>95	75-95	50-57	<50
Cost (US $/m^3)	>200	100-200	50-100	<50

2.5 Microbial Enhanced Oil Recovery (MEOR)

The use of biosurfactants for different purposes including oil recovery has taken a centre stage in the recent years because they are renewable, biodegradable and environmentally friendly (Gudina et al., 2015). Biosurfactants are applied for different purposes in the oil industry but the commonest applications include recovery of oil from oil wells, emulsion facilitated transport, cleaning of oil tanks, emulsion-based fuel production and environmental remediation (Dastgheib et al., 2008, Batista et al., 2006).

Dastgheib et al. (2008) argues that *Bacillus licheniformis* can be used for insitu MEOR because of its halo-tolerant properties. A wide range of different hydrophobic substrates, such as aliphatic or aromatic hydrocarbons can be emulsified efficiently to 65 ± 5 % by the lipopeptide surfactant produced by *Bacillus licheniformis.* With the halo-thermal properties of the genus bacillus, its strains accompanied with nutrients can be inoculated in the oil well for the in situ MEOR (Gudiña et al., 2012). The in-situ growth of microorganisms in MEOR can be hindered by the lack of sulphur, phosphorus and nitrogen in petroleum hydrocarbons much as there is presence of carbon and hydrogen (Cameotra and Singh, 2008). Biosurfactants have great surface and interfacial reduction capabilities which makes them a high priority in enhancement of oil recovery and demulsification processes (Desai and Banat, 1997). Indeed, when lipopeptide biosurfactant produced by the strain *Bacillus subtilis* was introduced to motor oil contaminated soil, it recovered 85 % of the oil in 24 hr (Bezza and Chirwa, 2015). When Gudina et al. (2015) used rhamnolipids in comparison to enordet and petrostep (synthetic surfactants) to recover crude oil from sand, the observed that rhamnolipids recovered 55 % of the crude oil while enordet and petrostep recovered 54.4 % and 30.5 % respectively.

2.6 Surfactants

A surfactant is an amphiphilic compound with a molecule consisting of a hydrophilic head and a hydrophobic tail (Mulligan, 2009). Besides offering the ability of organic pollutants to be removed from solid matrices such as soil in a fast process and a cost-effective manner, surfactants also offer the ability of a large volume of contaminated matrices to be treated (Boulakradeche et al., 2015). When surfactants are applied for remediation of hydrocarbons, the mobility of PHCs is enhanced by the hydrophobic tail when it makes the surfactant molecule to gather at the interfaces thereby reducing the interfacial or surface tension while the hydrophilic head increases PHCs solubility by allowing the surfactant molecule to dissolve in the water phase (Mulligan, 2009).

2.6.1 Types, characteristics and properties of surfactants

Surfactants can be produced chemically or biologically that's to say synthetic surfactants and biosurfactants respectively. Surfactants are classified into four groups as anionic, neutral/non-ionic, cationic and zwitterionic/amphoteric depending on the electric charge in their molecules (Cameselle et al., 2013, Boulakradeche et al., 2015, Volkering et al., 1998). The charge of most biosurfactants are dependent on the carboxyl groups with a pKa less than the pH of the solution in which they are made (Makkar and Rockne, 2003). Surfactants are classified as surface-active and emulsifying agents with the ability to reduce interfacial and surface tension between solids, liquids, and gases which allows them to readily disperse or mix as emulsions in other liquids (Singh and Cameotra, 2004). Brij 35, Igepal CA-720, Sodium dodecyl sulfate (SDS), Tween 80 and Tergitol are some of the most commonly used synthetic surfactants (Cameselle et al., 2013). Due to very unique characteristics of chemical surfactants they may be required to be applied as mixtures which is often done my mixing non-ionic and ionic surfactants to increase their effectiveness unlike biosurfactants (Makkar and Rockne, 2003).

Like already indicated a surfactant is an amphiphilic molecule in nature consisting of a hydrophobic tail (water-fearing) and a hydrophilic head which is water loving (Mulligan, 2009). The hydrophobic moiety is made of hydroxyl fatty acids, long-chain fatty acids, or α-alkyl-β-hydroxyl fatty acids while the hydrophilic moiety can be a phosphate, an alcohol, cyclic peptide, amino acid, carbohydrate, or carboxylic acid (Bezza and Chirwa, 2015, Wang et al., 2007). But the most commonly used hydrophobic parts of synthetic surfactants are alkylbenzenes, alcohols, olefins paraffins and alkylphenols and while the most common hydrophilic group is usually a quaternary ammonium group, carboxylate group, sulphonate group, sulphate group, polyoxyethylene, polypeptide and sucrose (Volkering et al., 1998).

On the other hand, the major classes of biosurfactants with the ability to exist as either particulate structures, single macromolecules or polymeric structures chemically exist as lipopeptides/lipoproteins, phospholipids, fatty acids and glycolipids (Makkar and Rockne, 2003). Biosurfactants generally have the lipophilic portion which is usually an alkyl tail. This may be unsaturated, hydroxylated saturated or branched and terminated by an acidic group. The hydrophilic group in this case is linked to the hydrophobic fatty acid by an amide bond or a glycosidic ester (Makkar and Rockne, 2003). An example is rhamnolipids that are made up of rhamnose sugar molecule and β-hydroxyalkanoic acid but they either manifest as di or mono rhamnoses attached to a β-hydroxyalkanoic acid in different mixtures especially during production (Wang et al., 2007). It has a great number of different congeners resulting from a significant variation of lengths of its fatty acids ranging from C_8 –C_{14}, as well as of 12- or 14-carbon chains with a single double bond (Wang et al., 2007). Because of these properties, rhamnolipids can greatly increase the emulsification of recalcitrant compounds such as petroleum hydrocarbons (Abalos et al., 2004, Wang et al., 2007). This was indeed reported by Abalos et al. (2004) who after

adding rhamnolipids observed an increase in the biodegradation rate of petroleum hydrocarbons from 32 % to 61 % after 10 days of incubation. The degradation included isoprenoids and PAHs with elevated levels of degradation ranging between 16 % to 70 % and 9 % to 44 % respectively.

The chemical and synthetic components of chemical surfactants have had them rendered environmentally unfriendly because of their toxicity and resistance to biodegradation (Mulligan et al., 2001, Gudina et al., 2015). On the other hand biosurfactants have been rendered a better substitution for synthetic surfactants because of greater environmental compatibility, highly biodegradability, high foaming capacity, low toxicity, higher selectivity, able to function at extreme pH, temperature, salinity but they can also be synthesized from a number of carbon containing substances (Bezza and Chirwa, 2015, Abalos et al., 2004, Wang et al., 2007, Desai and Banat, 1997). This same conclusion was made by Lima et al. (2011) who made an acute toxicity comparison between five bacterial surfactants and the synthetic surfactant sodium dodecyl sulphate on the bioluminescent bacterium Vibrio fischeri. They reported that biosurfactants had significantly lower toxicity than sodium dodecyl sulphate. This was mainly because the cell membranes of Vibrio fischeri were disrupted due to interactions with lipid components and by reacting with molecules with proteins essential to the functioning of the cell (Lima et al., 2011). Cationic surfactants are the most toxic in highly alkaline conditions while anionic surfactants are the most toxic under low pH or acidic conditions (Volkering et al., 1998). In the degradation of synthetic surfactants, it is most likely that they can produce more toxic intermediates compared to the pollutants being remediated (Volkering et al., 1998).

2.6.2 Biosurfactants and biosurfactant producing species

Biosurfactants are synthesised by a variety of microorganisms including bacteria, filamentous yeasts and fungi (Gudina et al., 2015). A variety of bacteria such as *Enterobacter, Acinetobacter, Pseudomonas, Arthrobacter, Halomonas, Bacillus, and Rhodococcus are also well* known for producing biosurfactants of which emulsans of Acinetobacter spp and the group of rhamnolipids produced by *Pseudomonas* are the most studied (Abalos et al., 2004). Table 2.2 below shows biosurfactants produced by a variety of bacteria. Biosurfactants are majorly classified as, lipopeptides, neutral lipids, polymeric or particulate compounds, glycolipids, fatty acids and phospholipids as shown in Table 2.2 (Bezza and Chirwa, 2015, Dastgheib et al., 2008, Wang et al., 2007, Singh and Cameotra, 2004, Desai and Banat, 1997). Bacteria are acknowledged for producing low molecular substances such as lipopeptides and glycolipids with the ability to reduce surface tension (Table 2.2) and high molecular weight polymers such as emulsan, biodispersan, alasan, or liposan with the ability to emulsify (Calvo et al., 2008, Ron and Rosenberg, 2002, Dastgheib et al., 2008). Some low molecular weight biosurfactants are formed when carbohydrates get attached to aliphatic acids or long chain lipopeptides and some are simply trehalose lipids, sophorolipids, or glycolipids made up of disaccharides that are acylated with hydroxy fatty acids or long-chain fatty acids. Most high molecular weight biosurfactants are majorly made up of proteins, lipopolysaccharides, lipoproteins, polysaccharides or complex mixtures of these biopolymers (Ron and Rosenberg, 2002). The lower molecular weight biosurfactants are reported to be efficient at lowering surface and interfacial tensions while high molecular weight biosurfactants are ineffective in doing the same but prevent the coalescence of oil droplets since they have the ability to tightly bind to the oil droplet surfaces (Ron and Rosenberg, 2002). This, therefore, means that as much as biosurfactants are known to have the ability to emulsify and reduce surface tension, bioemulsifiers

do not necessarily exhibit surface tension reduction properties (Batista et al., 2006). For example, bioemulsifiers have the ability to stabilise oil in water at very efficient levels but the same may not necessarily be said for their surface tension reduction levels (Dastgheib et al., 2008).

The length of the fatty acid, the congener specifications or any other specification of a biosurfactant classification is dependent on the bacterial strain, the biosurfactant production method or the carbon source used (Wang et al., 2007). Examples are *Pseudomonas* species that produce rhamnolipids, *Rhodococcus*, *Arthrobacter and Mycobacterium* species produce trehalolipids, *Bacillus subtilis* produce surfactin while *Candida and Torulopsis* produce sophorolipids (Calvo et al., 2008, Batista et al., 2006). With the great emulsifying ability, rhamnolipids have great surface tension reduction capabilities ranging from 72 mN/m to values below 30 mN/m and oil/water interfacial reduction capacities ranging from 43 mN/m to 1 mN/m (Wang et al., 2007, Volkering et al., 1998).

Bacteria can survive and grow under wide range of conditions such as temperature ranging from 25 to 55 ^{o}C and pH ranging from 6.5 to 8.5 (Das and Mukherjee, 2007). An example is *Pseudomonas aeruginosa* that has an optimum growth temperature of 45 ^{o}C and a pH of 7 while *Bacillus subtilis* has an optimum temperature of 55 ^{o}C and a pH of 8 (Das and Mukherjee, 2007). Gudiña et al. (2012) also observed that *Bacillus subtilis* strains grew at temperatures between 40 ^{o}C and 55 ^{o}C in solid medium with NaCl concentrations of up to 100 g/l and still grew at 50 ^{o}C in a liquid medium. Similar observations were not seen for the *Pseudomonas aeruginosa* as the strains failed to grow at temperatures above 42 ^{o}C in either of the same liquid or solid medium.

2.6.3 Screening of biosurfactant producing species

The screening of bacteria for biosurfactant production can be achieved by determining biomass concentration and biosurfactant producing after incubation

of the bacterial isolates at 40 ºC and 120 rpm (Gudiña et al., 2012). The most direct method for screening biosurfactant producing isolates is by the measurement of surface tension and emulsifying activity (Liu et al., 2012, Gudiña et al., 2012). The production of biosurfactants by specific isolates can be confirmed if the isolate can exhibit a reduction of surface tension below 40 mN/m (Batista et al., 2006, Gudiña et al., 2012, Desai and Banat, 1997). This method is however known for being time-consuming where there is a need to assess a large number of isolates (Liu et al., 2012). On the other hand, bioemulsifiers or biosurfactant producing microbes can also be identified if their supernatant or cells can maintain at least 50 % of an original emulsion 24 hours after it was formed (Batista et al., 2006, Desai and Banat, 1997). The emulsion stabilising test can be done by mixing 2 mL of cell free supernatant (obtained by centrifugation of an aliquot incubated at 28 ºC on rotary shakers at 150 rpm for 5 minutes at 12000 g) with 2 mL of kerosene in a test tube of known dimensions such as 100 mm x 15 mm. This is followed by shaking for 2 min and then left to stand (Batista et al., 2006, Hassanshahian et al., 2012). The relative emulsion volume (EV, %) and stability (ES, %) can be then measured at different intervals using the following formulae shown in equation (2.1) and (2.2) (Batista et al., 2006):

$$\text{EV, \%} = \frac{emulsion\ height\ (mm) \times cross\text{-}section\ area\ (mm^2)}{total\ liquid\ volume\ (mm^2)} \quad (2.1)$$

$$\text{ES, \%} = \frac{\%EV;\ \%\ at\ time\ t;\ h}{EV;\ \%\ at\ 0\ h} \times 100 \quad (2.2)$$

In contrast to the measurement of surface tension, Liu et al. (2012) suggest the use of blood agar lysis as a simple easy method to test for biosurfactant activity. After incubation of agar plates inoculated with microbes at about 37 ºC for 48 hours, a biosurfactant producing colony can be identified by the existence of hemolytic zone around the colonies. The two methods including surface tension

measurement and use of blood agar lysis can both be used in order to verify the results (Liu et al., 2012).

Screening of biosurfactant producing colonies can also be done by use of the drop collapse test (Batista et al., 2006). To screen using this method 2 µL of oil is applied in specified wells of a 96 well microtiter plate and left to equilibrate for 24 h. 5 µL of isolates that have been incubated for seven days at 28 ºC, shaken at 150 rpm and centrifuged at 12,000 g for 5 minutes to remove cells are transferred to the oil-coated well regions. The process is finalised by observing the drop size 1 min later with the aid of a magnifying glass to conclude on the results. If the drop diameter is larger (usually 1 mm larger) than that produced by deionised water the result is considered positive (Batista et al., 2006, Bezza and Chirwa, 2015).

Desai and Banat (1997) report that biosurfactant screening can also be done by use of the axisymmetric drop shape analysis (ADSA) which is done by placing drops of known volumes of culture broth on the surfaces of fluoroethylene-propylene and the droplet profile followed by determination of the profile with a contour monitor. It is also reported that calorimetric methods which utilise cationic indicators to form a coloured complex can be used by reacting the cationic indicator with anionic biosurfactants (Desai and Banat, 1997).

2.6.4 Identification of biosurfactant producing species

Cameotra and Singh (2008) suggest that biosurfactant producing species like other microorganisms can be identified and characterised according to their chemotaxonomic, morphological, and biochemical features.

The most commonly used method for identification and classification of biosurfactant producing bacteria is the use of the 16S ribosomal RNA (rRNA) sequencing. 16S ribosomal RNA (rRNA) identifies the isolates by analysing the taxonomy and phylogeny of the strain followed by depositing the sequence to the

Gen Bank data base to make the specific identification of the microbe (Gudiña et al., 2012, Bezza and Chirwa, 2015, Abalos et al., 2004). This involves the isolation of the total DNA from the cells followed by lysis with sodium dodecyl sulphate and treatment with cetyltrimethylammonium bromide (Gudina et al., 2015). The nucleotide sequences of the PCR product are then determined in both directions using universal primers by a DNA sequencing kit. The 16S rRNA gene sequences are then aligned with published sequences from the GenBank database (Abalos et al., 2004). The microbes can also be characterised by comparing their distinctive fatty acid gas chromatography profiles to the Microbial Identification System (MIS) data base (Cameotra and Singh, 2008).

Table 2. 2: The microorganisms responsible for producing specific types of biosurfactants as taken from Das and Chandran (2011), Uzoigwe et al. (2015) and Souza et al. (2014)

Microorganisms	**Biosurfactants**	**Biosurfactant classification**	**Surface tension (mN/m)**	**Interfacial Tension (mN/m)**	**CMC**
Bacillus subtilis	Surfactin,	Lipopeptides and lipoproteins	27-32	1	23-160
	Subtilisin	Lipopeptides and lipoproteins	-	-	-
Bacillus polymyxa	Polymyxins	Lipopeptides and lipoproteins	-	-	-
Bacillus licheniformis	Peptide-lipid	Lipopeptides and lipoproteins	27	0.1-0.3	12-20
Mycobacterium sp.	Trehalolipids	glycolipids	38	15	0.3

N. erythropolis	Trehalolipids	glycolipids	30	3.5	20
Pseudomonas aeruginosa	Rhamnolipids	Glycolipids	29	0.25	
	Protein PA	Polymeric surfactants	-	-	-
Pseudomonas Sp.	Rhamnolipids	Glycolipids	25-30	1	0.1 -10
Candida bombicola	Sophorolipids	Glycopilipids	-	-	-
Pseudomonas fluorescens	Rhamnolipids	Glycolipids	26.5	-	150
	Carbohydrate -protein-lipid	Polymeric surfactants	27	-	10
	Viscosin	Lipopeptides and lipoproteins	26.5	-	150
C. lepus	Fatty acids	Fatty acids, neutral lipids, and phospholipids	30	2	150
N. erythropolis	Neutral lipids	Fatty acids, neutral lipids, and phospholipids	32	3	-
A. calcoaceticus	Emulsan, biodispersan	Polymeric surfactants	-	-	-
C. lipolytica	Liposan	Polymeric surfactants	-	-	-
T. bombicola	Sophorolipids	glycolipids	33	1.8	-
T. apicola	Sophorolipids	glycolipids	30	0.9	-

A. calcoaceticus	Vesicles and fimbriae	Particulate biosurfactants	-	-	-

2.6.5 Biosurfactant production

The need for a cost-effective process of biosurfactant production to fulfil the current industrial demands can be reduced by optimisation (Batista et al., 2006). Production of biosurfactants under growth-limiting conditions, production by immobilised cells, production with precursor supplementation and growth associated production are the commonest mechanisms of fermentative biosurfactant production (Desai and Banat, 1997).

There exists a direct relationship between utilisation of substrates, cell growth and production of biosurfactants in growth associated production of biosurfactants; in growth limited production, nitrogen and iron sources are limited in the growth medium to control growth and prolong the existence of microbes in the stationary phase were maximum biosurfactant can be achieved; In immobilised production, cell multiplication is rested so that the existing microbes can utilise the available substrate leading to maximum biosurfactant production; lipophilic substances are added to the growth medium in production by precursor supplementation to stimulate maximum production of biosurfactants (Rodrigues et al., 2006, Desai and Banat, 1997). Production under growth-limiting has been reported for production of bio-surface active substances by *Nocardia sp, Candida tropicalis*, and *Torulopsis apicola*. Immobilized cell production has been used in production by *Pseudomonads, Torulopsis bombicola, Candida apicola, Rhodococcus erythropolis, Ustilago maydis,* and *Candida antarctica* while Production by precursor supplementation has been reported for production by *T. magnoliae, T. bombicola, and T. apicola* all resulting into increased biosurfactant production *(Desai and Banat, 1997, Rodrigues et al., 2006).*

2.6.6 Factors affecting biosurfactant production

Biosurfactant production is highly affected by the carbon source, oxygen availability, culture growth medium, agitation, and the surrounding growth conditions such as temperature, pH and salinity (Batista et al., 2006, Desai and Banat, 1997, Fadhile Almansoory et al., 2017). The carbon source used in biosurfactant production can affect the composition of the biosurfactant usually regarding the chain length (Desai and Banat, 1997). Different organisms have different preferences for the nitrogen source to achieve the highest production (Fadhile Almansoory et al., 2017).

Numerous carbon and nitrogen sources including crude oil, glycerol diesel, glucose, gasoline and sucrose as carbon sources and yeast extract, peptone, ammonium nitrate, and ammonium sulphate have been used as nitrogen sources (Fadhile Almansoory et al., 2017, Batista et al., 2006). Nitrogen and carbon sources affect the quantity and the quality of biosurfactants produced (Fadhile Almansoory et al., 2017). Nitrates have been reported to achieve maximum biosurfactant production by *Rhodococcus spp and Pseudomonas aeruginosa* while ammonium salts and urea have been reported as preferred nitrogen sources to produce biosurfactants by *Arthrobacter paraffineus* (Desai and Banat, 1997, Rikalovic et al., 2012). These differences were also observed by Batista et al. (2006) who selected 19 pure cultures and of these 17 tested positive for the production of biosurfactants when glucose was used as the carbon source; 2 tested positive when grown on sucrose and all the 19 isolates tested negative when they were grown on fructose as the sole carbon source. Similarly, environmental factors including pH affect different organisms differently including *Pseudomonas spp.* which produces maximum biosurfactant at a pH ranging from 6 to 6.5 and decreases sharply above pH 7 (Trummler et al., 2003, Desai and Banat, 1997).

On the other hand, temperature above 40 ºC is reported to cause alteration in the composition of the biosurfactant but the same temperature can positively influence production of biosurfactant in thermophile bacteria (Desai and Banat, 1997). Contrary to the conditions for biosurfactant production by Pseudomonads, when yeast extract was used as a substrate in the production of a bioemulsifiers from *Bacillus licheniformis* to determine the effect of temperature in the range of 15 to 60 ºC, salinity in the range of 0 to 8 % w/v of NaCl and pH in the range of 5 to 10, Dastgheib et al. (2008) observed that the bacterium produced the highest lipopeptides under optimum temperature of 45 ºC, NaCl concentration of 4 % (w/v) and pH of 8.0. Gudiña et al. (2012) also argue in agreement with a number of other authors that biosurfactant production by the microbes is highest in aerobic conditions as compared to anaerobic conditions. It's only a few halo-thermal tolerant strains such as those under the genus *Bacillus* that have the ability to produce biosurfactants under both anaerobic and aerobic and conditions (Gudiña et al., 2012). It should, however, be noted that heat hardly brings about any substantial change in the interfacial tension and surface tension properties of the biosurfactant even as high as 120 ºC (Desai and Banat, 1997, Bezza and Chirwa, 2015, Gudiña et al., 2012).

Salts have also been reported to reduce critical micelles concentrations with concentrations above 10 % but are generally stable in high saline conditions (Desai and Banat, 1997, Bezza and Chirwa, 2015). In agreement with other authors, Gudiña et al. (2012) also concedes that the increase in salinity increases the surface tension reduction potential of the biosurfactants as opposed to low salinity. This because in their studies maximum surface tension values were obtained at a pH of 6, with biosurfactants showing more stability in alkaline conditions with surface tension increasing below the pH of 5 due to precipitation of the biosurfactant.

2.6.7 Recovery, purification and characterisation of biosurfactants

2.6.7.1 Biosurfactant recovery and purification

Before choosing a biosurfactant recovery strategy, water solubility, the ionic charge, and the location are some of the factors that should be considered (Desai and Banat, 1997). But in general, ammonium sulfate precipitation is commonly reported for recovery of emulsan and biodispersan bioemulsifiers; acid and acetone precipitation recovers surfactin, Extraction using solvents has been found effective in recovering liposan, trehalolipids and tophorolipids; Glycolipids and cellobiolipids have also been recovered by crystallisation (Desai and Banat, 1997, Sriram et al., 2011, Pereira et al., 2013, Cameotra and Singh, 2008). Adsorption, continuous mode centrifugation, tangential flow filtration, ultrafiltration, and foam precipitation are some of the other recovery alternatives available (Desai and Banat, 1997).

The procedure of recovering biosurfactants by acid precipitation starts with the removal of the bacteria cells by centrifugation at around 10,000 rpm for 10 to 20 minutes at 4 ^{o}C. The cell-free supernatant is adjusted to pH 2 with 6 M HCl acid and left to stand overnight at 4 ^{o}C. The result is a precipitate which is collected by centrifugation at around 10,000 rpm for 10 to 20 minutes at 4 ^{o}C. The precipitate is then washed by deionised water at pH 2 and dissolved in deionised water to pH 7 (Liu et al., 2012, Gudiña et al., 2012, Bezza and Chirwa, 2015, Abalos et al., 2004).

2.6.7.2 Biosurfactant characterisation

After the recovery of biosurfactants from the growth medium, the biosurfactant can thereafter be analysed to determine its composition by Fourier transform infrared spectroscopy (FTIR), liquid chromatography–mass spectrometry (LC-MS), high performance liquid chromatograph-mass spectrometry (HPLC-MS),

thin layer chromatography (TLC) among others (George and Jayachandran, 2013, Rikalovic et al., 2012).

The chemical groups of the biosurfactants can be obtained by use of a Fourier transform infrared spectroscopy (FTIR) equipped with an Attenuated Total Reflectance. The result is a spectrum obtained as averages of 32 scans over a specified wavelength (Rikalovic et al., 2012). Thin layer chromatography may also be used to identify some compounds such as amino acids and sugar moieties. A pink pigment is revealed on the silica gel column chromatograms in the presence of amino acids after spraying with ninhydrin while the presence of sugar moieties can be determined by spraying with p-anisaldehyde (Bezza and Chirwa, 2015, Cameotra and Singh, 2008, George and Jayachandran, 2013). The Direct-injection mass spectrometric method and high-performance liquid chromatography (HPLC)-electrospray tandem mass spectrometry can be used for the rapid identification of the biosurfactant congeners obtained from the growth medium (Cameotra and Singh, 2008). Bezza and Chirwa (2015) suggest that the LC-MC analysis can be performed using a Waters acquity ultra performance liquid chromatography system coupled to a Waters Synapt G2 mass spectrometer to identify and quantify the chemical constituents of bio-surface substances.

2.6.8 Critical Micelle Concentration (*cmc*)

The critical micelle concentration is the concentration of the biosurfactants (or any other amphiphilic compounds) above which micelles start forming (Gudiña et al., 2012, Batista et al., 2006, Desai and Banat, 1997). At critical micelle concentration, the surfactant brings about variations in the physicochemical interactions between the surfactant and the solution (Makkar and Rockne, 2003). Stable micelles are formed when aggregation of molecules between 10 to 200 molecules occurs (Makkar and Rockne, 2003, Volkering et al., 1998). The formation of the micelles is mainly dependant on the surfactant concentration and temperature (Cameselle et al., 2013, Volkering et al., 1998). The *cmc* can be

determined by plotting the surface tension as a function of the logarithm of biosurfactant concentration. The *cmc* is, therefore, the intersection of two straight lines traced through plots of the measured property versus the surfactant concentration (Gudiña et al., 2012, Batista et al., 2006). Surface tension values can be obtained by determination of the surface tensions using the ring method at room temperature with the pH of the samples adjusted to 7 (Gudiña et al., 2012).

2.7 Bacteria and biosurfactants in bioremediation

A total of 79 bacteria, 103 fungi, 14 algae, and 9 cyanobacteria species have so far been identified as known potential hydrocarbon degrading organisms from the numerous studies done by researchers (Hassanshahian et al., 2012). Table 2.3 below shows some of the bacteria that can degrade hydrocarbons. The biodegradation of hydrocarbon containing compounds entirely depends on the amount and nature of the hydrocarbons available (Das and Chandran, 2011). It is possible to achieve almost 100 % degradation using marine bacteria and almost 50 % with soil bacteria (Das and Chandran, 2011). Besides being rich in biodiversity and having the ability to adapt to different environments in different ways such as modifying the cellular membranes, bacteria produce biosurfactant and possess catabolic genes and enzymes that strongly diversify and intensify their catabolic potential (Tyagi et al., 2011, Makkar and Rockne, 2003, Das and Chandran, 2011, Desai and Banat, 1997). The most commonly studied bacteria in petroleum hydrocarbon bioremediation processes are seen in Table 2.3 below as derived from (Tyagi et al., 2011, Chauhan et al., 2008, Das and Chandran, 2011).

Table 2. 3: Bacteria used in petroleum hydrocarbon bioremediation processes

Bioremediated hydrocarbon	**Species**
Aromatic hydrocarbons e.g toluene, ethylbenzene, benzene, xylene etc	*Rhodococcus, Pseudomonas, and Ralstonia*
Plasmid Naphthalene	*Pseudomonas*
Phenanthrene	*Haemophilus and Pseudomonas species*
Polyromantic hydrocarbons such as naphthalene	*Pseudomonas*
Pyrene	*Haemophilus and Mycobacterium*
Anthracene	*Rhodococcus*
Highly carcinogenic benzo[a]pyrene	*Mycobacterium and Rhodococcus*
Salicylate	*Pseudomonas*
C_1-C_8 alkenes, alkanes and cycloalkanes	*Methylosinus, Methylomonas, Methylococcus, Methylocella, Methylocystis*
C_5–C_{16} fatty acids, alkanes, cycloalkanes, and alkyl benzenes	*Mycobacterium, Pseudomonas, Candida tropicalis, Yarrowia lipolytica, Rhodococcus, Burkholderia, Candida maltose, Acinetobacter and Caulobacter*
C_{10}–C_{30} alkanes	*Acinetobacter sp.*

Amidst plenty of studies made to evaluate the influence of biosurfactants on the biodegradation of compounds such as such octadecane, phenanthrene or fluoranthene, Abalos et al. (2004) claims that mixed cultures are more effective in degrading most of these compounds than pure cultures due to the recalcitrant

properties and complexity of hydrocarbons. This is because mixed cultures can achieve broader enzymatic capacity and the possible counteraction to the anticipated toxic metabolites by a meticulous choice of known counteracting microbe degraders (Abalos et al., 2004). It is also acknowledged that much as different petroleum hydrocarbons degrade at different rates, 70-97 % of those in crude oil are biodegradable with more than 70 genera of microorganisms having the ability to degrade them (Prince et al., 2003). It is logically sound to use biosurfactant producing hydrocarbon degrading microbes than to apply biosurfactants to enhance bioavailability for non-biosurfactant producing microbes (Cameotra and Singh, 2008). Das and Chandran (2011) claims that Pseudomonas species have the greatest capability of degrading hydrocarbons and producing biosurfactants (Das and Chandran, 2011). n-alkanes are the most biodegradable PHC's while those with 5 to10 hydrocarbon atoms are the least biodegradable because they inhibit the functionality of degrading microorganisms by damaging or distorting their lipid membranes (Tyagi et al., 2011). The C_{20}-C_{30} waxy PHC's are also hardly biodegraded because of their hydrophobicity properties explaining the decreasing order of biodegradability from n-alkanes to branched-chain alkanes to branched alkenes to low molecular-weight n-alkyl aromatics, monoaromatics, cyclic alkanes, polycyclic aromatic hydrocarbons (PAHs) to asphaltenes as the most recalcitrant (Tyagi et al., 2011, Das and Chandran, 2011). The kinetic modelling for the degradation of hydrocarbons can be done by considering the biodegradability of the hydrocarbon using Monod's first order kinetics taken from Zahed et al. (2011) as shown in equation (2.3) below;

$$C = C_o e^{-kt} \tag{2.3}$$

Where C is the concentration of hydrocarbons (g/kg) at time t (day), C_o is the initial concentration of hydrocarbons (g/kg) and k is rate constant of the change in the hydrocarbon content (day^{-1}). The unknown concentration of the

hydrocarbons at given time t of microbial degradation can obtained by plotting a logarithm of hydrocarbon concentration against time (Zahed et al., 2011).

2.7.1 The processes involved in the biodegradation of organic compounds

The biodegradation mechanism starts with an entirely aerobic process by intracellular attack of hydrocarbon pollutants followed by enzymatic activity. Organic pollutants are then converted into intermediates of the central intermediary metabolism (Das and Chandran, 2011). An example are alkanes which are major components of crude oil known to be degraded by an enzyme called alkane hydroxylase (Hassanshahian et al., 2012). Alkane hydroxylase starts off the process by deriving an oxygen atom from molecular oxygen to introduce it into the alkane substrate. Amongst the three Alkane hydroxylase genes, alk-B genes (group I) catalyse the short-chain n-alkanes (C_6–C_{12}), alk-M genes (group II) catalyse the degradation of medium-chain n-alkanes (C_8–C_{16}) and alk-B genes (group III) catalyze the degradation of long-chain n-alkanes (>C_{16}) (Hassanshahian et al., 2012).

2.7.2 Factors affecting degradation of hydrocarbons

The degradation of hydrocarbons is inhibited by low bioavailability of the substrate (hydrocarbons) to the microorganisms due to their recalcitrant and hydrophobicity properties. The reduced level of bioavailability of the hydrocarbons to the bacteria is because of the tendency of the petroleum hydrocarbons to bind to the soil compounds making it so difficult to be degraded (Das and Chandran, 2011, Volkering et al., 1998). Bioavailability is reported to be dependent on the general mass transfer and movement of the pollutant into the aqueous bulk phase (Volkering et al., 1998). Biosurfactants are reported to have the ability of increasing the bioavailability of PAH's and the apparent solubilities of PAHs 5–20-fold hence significantly increasing their rate of biodegradation (Chauhan et al., 2008). This is because biosurfactants reduce surface tensions and

interfacial tensions (Batista et al., 2006). Biosurfactants have been reported to be more effective in improving the bioavailability of hydrophobic substances as compared to the chemical surfactants (Ron and Rosenberg, 2002). Unlike synthetic surfactants which may necessitate the use of different surfactant-utilizing and PAH-degrading strains together to avoid inhibition of the degradation process, a single bacteria culture can be used to simultaneously produce the biosurfactant and degrade the PAH's (Chauhan et al., 2008, Makkar and Rockne, 2003). It has indeed been previously observed that the introduction of exogenously produced biosurfactants into hydrocarbon polluted soils enhances the bioremediation process by the indigenous microbes (Bezza and Chirwa, 2015, Cameotra and Singh, 2008, Das and Mukherjee, 2007, Batista et al., 2006). By reducing surface tension and interfacial tensions biosurfactants increase the surface area of oil which making it accessible to the available microorganisms (Das and Chandran, 2011).

When surfactants are applied, they increase the availability of compounds to microbes through three interrelated mechanisms: the first mechanism only happens in the presence of nonaqueous-phase liquid organics and it involves the reduction of the interfacial tension between the aqueous and nonaqueous phase as a result of dispersion of nonaqueous-phase liquid organics. In the second mechanism the presence of micelles that contain high concentrations of the hydrophobic organic contaminants increase the solubility of the pollutant which lowers the surface tension of the soil particle pore water; and lastly the interaction of the pollutant with single surfactant molecules and interaction of the surfactant with solid interfaces enables the expulsion of the pollutants from the solid phase of the contaminated matrix (Makkar and Rockne, 2003).

Biosurfactants can stabilise or destabilise emulsions and this may be determined by how the biosurfactant produces a turbid emulsion mixture due to hydrocarbon suspensions or the demulsification effect on a stable emulsion (Desai and Banat,

1997). The use of biosurfactants in bioremediation entirely depends on their ability to enhance desorption, dissolution, and to stimulate biodegradation of the pollutants (Makkar and Rockne, 2003). It is reported that there is direct relationship between biosurfactant production, cell surface hydrophobicity, the emulsification activity and crude oil biodegradation. This means that highly cell surface hydrophobicity organisms which exhibit high biosurfactant production with high emulsification activity usually have the greatest petroleum hydrocarbon degradation (Hassanshahian et al., 2012). Bezza and Chirwa (2015) claim that they used lipopeptide biosurfactant produced by *Bacillus subtilis* strain to improve degradation of motor oil polycyclic aromatic hydrocarbon components up to 82 % in 18 days of incubation. Cameotra and Singh (2008) also reported that a mixture of 11 rhamnolipid congeners degraded 91 % of the organic content of soil contaminated with crude oil sludge in 5 weeks.

The interactions between the pollutant, the matrix, and the microorganisms are dependent on environmental factors (such as temperature, oxygen and nutrients), the type and physicochemical state of the pollutant, soil and the microorganisms (Volkering et al., 1998). A wide number of factors have been reported to influence hydrocarbon degradation including temperature and nutrients. Temperature can affect the solubility of the petroleum hydrocarbons either because of the increase or reduction of viscosity while on the other hand nutrients such as nitrogen, phosphorus and potassium can affect the level of degradation. Much as nutrients are quite important, it has reported that if they are high in quantity, they negatively affect the degradation of aromatics (Das and Chandran, 2011). The maximum levels of degradation can be achievable between 30 to 40 ^{o}C in the soil environment, 20 to 30 ^{o}C in fresh water and 15 to 20 ^{o}C in marine environments (Das and Chandran, 2011).

Degradation of short chain alkanes ranging from C_9 to C_{11} is inhibited due to its toxicity to the bacteria which dissolves the cellular membrane while the long

chain (C_{19}-C_{25}) degradation is inhibited by the fact that long-chain n-alkanes are solid with low solubility. The medium length alkane chains ranging from C_{12}-C_{18} are however highly degradable as compared to the other two (Hassanshahian et al., 2012).

Much as surfactants increase the availability of pollutants in bioremediation processes in general, there is also a possibility of increasing non-bioavailability in cases where the surfactants lead to the formation of micellar substrates (such as the formation of micellar PAH's) if these micelles are above the critical micelle concentration (Makkar and Rockne, 2003).

2.8 Electrokinetics

This phenomenon was first operated and observed by Reuss in the application of direct current in a clay-water mixture in the 19th century followed by an overview kinetic proposition of the likely processes by Helmholtz and Smoluchowski (Virkutytea et al., 2002). The electrokinetic method employs the use of direct current applied across an electrode pair (anode and cathode) placed on either side of a porous medium to cause electroosmosis of the liquid phase, electrophoresis of charged particles and electromigration of ions to oppositely charged electrodes (Yang et al., 2005, U.S.EPA, 1997, Altin and Degirmenci, 2005).

The electrokinetic process is also commonly referred to as electro-reclamation, electrokinetic remediation, electrokinetic soil processing and electrochemical decontamination of wastes (U.S.EPA, 1997, Acar et al., 1995, Xu et al., 2014). All these defer depending on whether the process is meant to remediate soil, sludge, slurries or groundwater to remove inorganic, organic, radioactive and heavy metal wastes (U.S.EPA, 1997, Acar et al., 1995, Xu et al., 2014).

The contaminants treated by this method include arsenic, cadmium, chromium, copper, lead, nickel, zinc, uranium, mercury, lead, volatile organic compounds, BTEX compounds (such as toluene, xylene, benzene, and ethylbenzene), phenols, polychlorinated biphenyls, toluene, chlorophenols, trichlorethane and total petroleum hydrocarbons (U.S.EPA, 1997, Acar et al., 1995, Virkutytea et al., 2002, Kim et al., 2014b). Elektorowicz et al. (2006) claim that the application of electrokinetics in oil-contaminated matrices for purposes of oil recovery and remediation can increase the demulsification rate by 200 %.

2.8.1 The fundamental theory of electrokinetics

They are mainly five processes that make up this phenomenon and these include electromigration diffusion, electrophoresis, electroosmosis, and electrolysis (U.S.EPA, 1997, Altin and Degirmenci, 2005). “Electro-osmosis is the

movement of the capillary water under the electric field due to the existence of the electrical double layer at the interface of water and the solid surface while electrophoresis is the migration of charged particles or ions in a colloidal system towards the counter charged electrode. The direction of the electro-osmotic flow is decided by the nature of the charges on the surface of solid particles, i.e. negatively charged particles resulting in water going towards cathode while positively charged particles making water move towards anode (Popov et al., 2008, Yang et al., 2005). Electrolysis describes the chemical reactions that occur at the electrodes while diffusion involves the distribution of the matter that lies in-between the electrodes (U.S.EPA, 1997). Acar et al. (1995) argues that the complementary combination of these five processes is what makes electrokinetic remediation a cost effective and technically feasible means of removing contaminants from soil and other solid matrices because the migration flux resulting from one of the processes would still transport the species to where they can be removed from the matrix.

Oil contaminated mediums form emulsions of medium constituents because of the presence of resins, waxes, asphaltenes, finely divided minerals and organic acids which are usually emulsifying compounds found in the oil. These substances together with fine particles in contaminated matrices in contact with oil and water form solid stable emulsions hence reducing the demulsification constant which makes it difficult to separate the constituents (Elektorowicz et al., 2006). The application of an electric field influences the separation of different phases in different ways. The separation of different phases (oil, water, and solids) in oil-contaminated soil mainly involves the electro coalescence of small oil and water droplets to attain aggregated droplets that can form separable liquid phases, the movement of charged colloidal particles from the contaminated matrix to the oppositely charged electrode and the electroosmotic flow of oil and water towards the cathode (Ali and Alqam, 2000). The main kinetic processes in

electrokinetic remediation are electromigration and electroosmosis (Cameselle et al., 2013, García-Rubio et al., 2011). Electromigration is responsible for the movement of ions and is therefore responsible for transportation of ionic metals, colloidal electrolytes, ionic micelles, and polar organic molecules. This happens with the movement of anions to the anode and movement of cations to the cathode (Cameselle et al., 2013). Electroosmosis on the other hand is dominant for the transportation of inorganic and organic contaminants (Cameselle et al., 2013). Depending on the polarity on the solid matrix, the flow tends towards the anode or cathode. In case of an electropositive solid matrix the electroosmotic flow tends towards the anode while the electroosmotic flow tends towards the cathode if the solid matrix is electronegative (Cameselle et al., 2013).

Several chemical reactions affecting the speciation of the contaminants occur in the electrokinetic cell when the electric field is applied. These may include redox reactions, acid-alkaline reactions, dissolution-precipitation reactions, and adsorption-desorption (Cameselle et al., 2013, Xu et al., 2014, Lu et al., 2012, Rutigliano et al., 2008). The disassociation of the contaminants in the matrix towards the electrode compartments is strongly dependant on compounds octanol-water partition coefficient and solubility (Guedes et al., 2014). The analysis, determination and modelling of the physico-chemical and electrochemical processes in an electrokinetic system is a very complex quest requiring the understanding of the balance of the pore fluid per unit volume in a pore medium, chemical speciation and transportation, adsorption of compounds on to colloids, distribution of the electric potential in the system and mass transfer in the electrolyte wells (López-Vizcaíno et al., 2017, Masi et al., 2017). But one of the most important reactions that happens at the electrodes is the decomposition of water. Oxidation reactions occur at the anode (equation 2.4) and reduction reactions occur at the cathode (equation 2.5) as seen below

(Cameselle et al., 2013, Shen et al., 2007, Guedes et al., 2014, Rutigliano et al., 2008, García-Rubio et al., 2011, Shu et al., 2015, Jeon et al., 2015).

$$2H_2O \rightarrow 4e^- + 4H^+_{(aq)} + O_{2(g)} \quad (2.4)$$

Reduction reactions occur at the cathode as seen below

$$4H_2O + 4e^- \rightarrow 2H_{2(g)} + 4OH^-_{(aq)} \quad (2.5)$$

These reactions lead to the formation of the alkaline front at the cathode and an acid front at the anode on the immediate application on an electric field but as ions start migrating, the pH dynamically changes across the system as the H^+ ions move towards the cathode. With H^+ almost twice mobile (1.75 times) as OH^- the protons dominate resulting into the movement of the acid front towards the cathode where H^+ ions meet OH^- ions and form water (Shu et al., 2015). The pH in the system is, therefore, dependant on the movement of H^+ and OH^- across the system (Cameselle et al., 2013, Xu et al., 2014, Giannis et al., 2010, Shu et al., 2015). H^+ ions lead to desorption of the pollutants from the absorbent such as soil but also lead to the dissolution of the pollutant in the system. On the other hand, OH^- ions lead to the precipitation of heavy metals in the cathode area due to an increase in pH thus reducing the efficiency of the system (Giannis et al., 2010, Lu et al., 2012). High ionic concentration decreases the electroosmotic flow to literally unmeasurable levels due to the increase in the thickness of the double layer which confines the electroosmotic flow of the pore fluid to the periphery of the capillary (Acar et al., 1995).

Electroosmosis comes to an end when the counteracting flux under the hydraulic gradient equals the electroosmotic fluid flux or, if the soil surface potential approaches zero charge as a result of changes in the pore fluid composition (Acar et al., 1995).

Though expensive to do, electrokinetics can be applied in combination with other methods such as chemical, oxidation and permeable reactive barriers in order to improve the efficiency of the system and prevent pH effects (Cameselle et al., 2013, Lu et al., 2012). Also referred to as the Lasagna Technology, permeable reactive barriers are commonly used in combination with the electrokinetic remediation processes for in-situ remediation processes (Roulier et al., 2000, Kimura et al., 2007b, Xu et al., 2016). Chelators are also used for this purpose as they tend to desorb the pollutants such as heavy metals (Xu et al., 2017, Popov et al., 2008, Kornilovich et al., 2005). Polarity exchange can also be one of the techniques to respond to precipitation of the contaminant without application of additional chemicals such as chelators which may lead to further contamination of the medium under treatment (Lu et al., 2012, Mena et al., 2016, Pazos et al., 2006). Elektorowicz and Hatim (2000) also argue that the separation of the phases during electrokinetic remediation of contaminated mediums may be affected by pH, the spacing of the electrodes resistance and electrical potential but suggest that the process could be improved by the use of surfactants to increase the efficiency of pollutants removal from contaminated matrices such as soil. Zhang et al. (2014) argue that the overall contaminant removal after the electrokinetic remediation process can be calculated using equation (2.6) as follows;

$$w = \frac{C_o - C_1}{C_o} \qquad (2.6)$$

Where C_o is the initial concentration of the contaminant, C_1 is the amount of the contaminant that remains after bio-electrokinetic treatment and w is the overall efficiency. It should however be noted that contaminant removal using electrokinetics is usually low without an enhancement (Cang et al., 2013).

2.8.2 Electrodes and electrolytes

The electrokinetic cell is basically made up of two electrodes and electrolyte chambers referred to as the cathode electrode with a catholyte as the electrolyte and the anode electrode with an anolyte as the electrolyte (Yang et al., 2005, U.S.EPA, 1997, Elektorowicz and Habibi, 2005). In remediation of oil-contaminated environments, electrode spacing affects the oil phase and water phase contents in the liquid phase differently (Yang et al., 2005). The larger the spacing the higher the rate of oil recovery while the lower the spacing the higher the dewatering efficiency (Yang et al., 2005). In the electrokinetic dewatering of oil sludge by Yang et al. (2005), they reported the highest dewatering efficiency at the lowest electrode spacing of 4 cm as compared to 6 cm and 8 cm while the highest oil recovery rate was reported for the highest electrode spacing of 8 cm as compared to 6 cm and 4 cm much as the differences where very small as compared to those of the dewatering process. This means that approaching electrodes have been used as an effective method to improve the rate of contaminant removal in the system (Li et al., 2012, Zhang et al., 2014).

Electrode materials affect voltage loss depending on their electrochemical potential; Materials with low electrochemical potential have been found to have a low electrode-electrolyte interface voltage loss and vice versa (Yang et al., 2005). This was reported by Yang et al. (2005) who observed a substantial loss of voltage at the interface of the anode made of carbon with an electrochemical potential of +1.18 V as compared to the cathode made of iron with an electrochemical potential of −0.44 V. This resulted into the generation of more heat in the anode compartment which increased operating temperatures of the whole system. Electrodes can produce ions into the system that may affect the efficacy of the process (Sawada et al., 2004). Having performed two different experiments using iron and graphite, Sawada et al. (2004) reported that production of Fe^{2+} ions affected the removal of lead by precipitating as $Fe(OH)_2$.

The introduction of new species to the system due to electrode-based reactions can be prevented by using inert electrodes. Inert carbon is a good example that has been used besides its low cost. Due to the high acidity in electrode compartments, high grade carbon should be used as the anode and the low-grade metals may be used as cathode electrodes (Acar et al., 1995). But where this is impossible, precipitation may also be prevented by introduction of processing, conditioning or complexing fluids. These fluids serve as a conducting media, enhance the transportation of system species, control modification and depolarisation of electrode reactions (Acar et al., 1995). The movement of these fluids across the electrodes enhances the desorption of species and the dissolution of carbonates and hydroxides (Acar et al., 1995, Park et al., 2009, Reddy and Shirani, 1996, Kim et al., 2009b, Zhang et al., 2014). Electrodes may also either inhibit or enhance the removal of pollutants by either decreasing or increasing electroosmotic flow or electric current flow (Yuan et al., 2016). In their experiments Yuan et al. (2016) reported a 30 % increase in the removal of cadmium, nickel, and zinc using carbon nanotube covered polyethylene terephthalate yarns electrodes as compared to Pt/Ti and graphite electrodes as a result of elevated electric current and electroosmotic flows in the system.

In in-situ electrokinetic remediation, great emphasis is put on the configuration of the electrodes besides consideration of voltage gradient, electrolyte and operational time because electrode configuration strongly affects the final efficiency of the process and the overall costs (Kim et al., 2014b). Approaching anodes as an electrode configuration strategy can decrease the pH, increase electromigration hence improving the process of remediation (Shen et al., 2007, Zhang et al., 2014). Approaching anodes can save the energy of the process by 44 % and time by 40 % where energy consumption per unit volume is as shown below in equation (2.7) (Shen et al., 2007, Yuan et al., 2016, Zhang et al., 2014,

Reddy and Saichek, 2004, Yuan and Chiang, 2008, Kim et al., 2014b, Jeon et al., 2015).

$$E_u = \frac{1}{V_S}\int VIdt \qquad (2.7)$$

Where V_S is volume of the medium such as soil, V is the voltage difference between the electrodes, and I is the electric current. E_u is calculated as kWh/m^3

Electrolytes affect electric current flow in the system depending on the rate of disassociated ions. The higher the concentration of mobile and transferrable ions generated from the electrolyte the higher the current (Zhu et al., 2015). Zhu et al. (2015) observed and reported higher current variations when ammonia water was used as compared to when deionised water was used due to the difference in concentration of mobile and transferrable ions. It has also been reported that high electrolyte concentration and low pore fluid pH can easily reverse the polarity of the surface charge hence initiating the electroosmotic flow towards the opposite direction (Acar et al., 1995).

2.8.3 Electroosmosis and its effects to the remediation process

Electroosmosis is affected by viscosity, ionic concentration, temperature, the dielectric constant of the interstitial fluid and surface charge of the solid matrix also known as the zeta potential (Cameselle et al., 2013, Rozas and Castellote, 2012, Virkutytea et al., 2002, Shu et al., 2015). The surface charge of the solid matrix such as soil may be permanent due to isomorphic substitution or maybe temporary and variable due to the desorption and sorption of hydroxide ions and hydrogen ions on the surface of the solid matrix (Park et al., 2009, Kim et al., 2009b). The direction in which the liquid phase (electroosmotic flow) flows is strongly influenced by the zeta potential sign much as this can be changed by the conditioning or chelating agents if the solid matrix possesses a temporary charge (Rozas and Castellote, 2012, Park et al., 2009, Guedes et al., 2014, Kim et al., 2009b, Baek et al., 2009). At low system pH the zeta potential is usually positive

and may influence electroosmotic flow towards the anode (Guedes et al., 2014, Baek et al., 2009). The higher the negativity of the zeta potential the higher the velocity of the electroosmotic flow towards the cathode (Altin and Degirmenci, 2005). The liquid phases separated (water and oil) after the introduction of an electric current move to the cathode in most cases as a result of electroosmosis but in some rear occasions the liquid phase ends more concentrated at the anode (Yang et al., 2005). This was reported by Yang et al. (2005) who observed a high oil and grease flow towards the anode as compared to the cathode. This kind of electroosmotic flow eliminated potential reactions between oil or grease and hydroxy ions in the cathode compartment that would have potentially resulted in the formation of soap which is obviously undesirable during the oil recovery process.

Electroosmosis decreases with a decrease in the pH of the system and a decrease in the density of the medium (Xu et al., 2014). High concentration of H^+ ions can decrease the rate of electroosmotic flow if they impose a net positive charge on the solid medium as a result of their concentrations (Xu et al., 2014, Ouhadi et al., 2010). It should also be noted that the application of chelating agents and conditioning agents can increase the efficiency of the system by increasing the electroosmotic flow (Reddy and Shirani, 1996, Kim et al., 2009a, Rozas and Castellote, 2012, Agnew et al., 2011, Kim et al., 2009b). Either catholyte or anolyte conditioning can be used to enhance the removal of the contaminant from the matrix by controlling the matrix pH (Kim et al., 2009b, Baek et al., 2009, Cang et al., 2013). Alkaline conditioning is however said to enhance the electroosmotic flow much greater than the acidic conditioning (Kim et al., 2009a, Baek et al., 2009). This was observed by Kim et al. (2009a) when anolyte conditioning was done with the application of alkaline solutions leading to improved fluorine removal of 75.6 % from contaminated soil.

Helmholtz–Smoluchowski's kinetic equation describes the relationship between the electric field applied and the electroosmotic flow. According to this equation there is direct proportionality between the electric field and electroosmotic flow implying that the higher the electric field applied the higher the electroosmotic flow in the system (Mena et al., 2016, Rutigliano et al., 2008). This relationship is shown in equation (2.8) below as adapted from Reddy and Saichek (2004), Mena et al. (2016) and Boulakradeche et al. (2015) where electric field is represented by E_x, electro-osmotic flux is EOF (m/s), dielectric constant is D, vacuum permittivity is ε_o, and fluid viscosity is μ;

$$EOF = \frac{-D\varepsilon_0 Z}{\mu} E_x \tag{2.8}$$

The process of electroosmosis can be affected by viscosity and the molecular size of the pore fluids such as water and oil. The larger the size of the molecules the lower the electroosmotic rate since the liquid phases may not easily go through the filter to the electrode chambers. This can affect the rate of oil recovery as opposed to dewatering as oil has larger molecules which means it's outcompeted by water which smaller molecules (Yang et al., 2005). This was observed by Yang et al. (2005) at the beginning of the electrokinetic process when no coalesced oil and grease could flow through the filter leading to its accumulation at the anode area. Oil flow only increased when the water flow rate decreased indicating an increase in more available filter pores.

2.8.4 Electromigration and its effects to the remediation process

Electromigration is affected by the porosity and conductivity of the solid medium, pH gradient, applied electric potential, grain size, pore water, current density, ionic mobility, initial concentration of the specific ions in the medium and the presence of competitive ions (Cameselle et al., 2013, Virkutytea et al., 2002). Xu et al. (2017) reports that the force (F) applied to the ions to induce their movement

is a product of charge of ionic species (Z_i), elementary charge (e =1.6 × 10^{-19} C) and voltage gradient (▽Vin V/cm) as shown in equation 2.9 below.

$$F = Z_i e \times \nabla V \quad (2.9)$$

It is a basic observation to have an electroosmotic flow of the liquid phase when an electric field is applied to the electrokinetic reactor but in instances where the ionic concentration is too high, there is a possibility of not observing measurable electroosmotic. Therefore, the concentration and transportation of charged species is as important as electroosmosis in electrokinetic remediation (Acar et al., 1995). Approaching electrodes have been used to improve electromigration thus increasing the flow of electric current which in turn improves the removal of the contaminant from the medium (Li et al., 2012). The dielectric constant of substances in the system is directly proportional to the dissociation of the ions in those substances which affect electromigration and the electric field flow in the system (Maturi and Reddy, 2008). The dielectric constant of water is 3 times that of non-aqueous substances which means that the current flow in water should be higher than that in non-aqueous substances such as cosolvents but this may be otherwise in cases where a lower number of ions in water participated in the electrolysis reaction (Maturi and Reddy, 2008). Under high pH, the organic aqueous interfaces are usually negatively polarised due to adsorption of hydroxyl ions majorly produced from the cathode area with the polarity of the oil contaminants highly related to the aromatic fraction which together with aliphatic hydrocarbons are constituents of its hydrophobic fraction (Elektorowicz et al., 2006). The rate of phase separation therefore strongly depends on the polarity of the hydrocarbon mixture (Elektorowicz et al., 2006).

2.8.5 Demulsification rate due to application of an electric current

The greatest problem related to the disposal and the separation of phases in the oil-contaminated slurries and sludge is the high-level emulsion which is affected by temperature, phase volume ratio, hydrophile-lipophile balance (HLB), agitation, electrical potential, electrolyte, wettability, interfacial tension, and viscosity leading to very tight, lose or stable emulsions depending on the crude oil constituents (Elektorowicz et al., 2006). Emulsions are a colloidal system of dispersed immiscible liquids in the form of small droplets (with dimensions in the range of 1 nm to 1 μm) in a continuous liquid phase. Oil emulsions can occur as O/W emulsions or W/O emulsions depending on which liquid is the continuous phase (Rocha et al., 2017).

The speed of emulsion separation, the quality of the disposable separated water, and the amount of the liquid (oil or water) left in the matrix after separation are the three most important aspects to consider in the demulsification of oil contaminated matrices (Rocha et al., 2017). The electro-demulsification process is visible when the breakdown of colloidal particles starts just following the application of an electric field. The colloidal particles move in the vertical direction amidst the movement of the pore fluid in the horizontal direction (Elektorowicz et al., 2006). The movement of the colloids is followed by their coagulation near the anode. Sedimentation of the particles increases as collisions between these particles increases. The rate of colloidal aggregation is greatly affected by the applied electrical potential. Slow coagulation of the particles produces compact particles while fast coagulation produces loose particles. Electrical potential high affects the electro demulsification process in an electrokinetic reactor. A lower electrical potential is most likely to produce a more efficient demulsification rate and phase separation (Elektorowicz et al., 2006). In their investigation to optimise the operation of the electrokinetic cell in phase separation, Elektorowicz and Habibi (2005) varied the voltages from 0.5 to

1.5 V/cm and concluded that a lower electrical potential generated the highest demulsification rate. Yang et al. (2005) observed a similar occurrence as the rate of dewatering from oil sludge increased with an increase in applied voltage from 10 V to 20 V while a further increase to 30 V led to efficiency reduction. According to Elektorowicz et al. (2006), demulsification rate can be expressed in terms of the height of the emulsion before (H_o) and after the experiment (H) with an overall demulsification rate constant (K_d), and duration of the experiment (t) as follows in equation (2.10).

$$\frac{H}{H_0} = \exp(-k_d t) \quad (2.10)$$

2.8.6 Current

Like pH, electric current in the system can be affected by the type of the solid matrix in the system such as the soil type in soil remediation (Xu et al., 2014). This is because the electric current in the system is affected by the conductivity of the medium, water content and the applied voltage (Xu et al., 2014).

Electric current in the system is expected to rise in the initial stages of the experiment reportedly 72 hours after the beginning of the experiment depending on the electrical conductivity of the medium and the resistance (Xu et al., 2014, Guedes et al., 2014). The high current values observed during the initial stages of the process are due to the high electromigration of ions in the system which continues until equilibrium is reached due to reactions between the ions and the compounds in the system (Pham et al., 2009). This could as well be related to the resistance of the matrix in the system due to reduction or depletion of mobile ions (Guedes et al., 2014).

A few hours after the beginning of the experiment current flow gradually reduces due to the loss of ionic strength in the pore fluid and resistance polarisation (Shen et al., 2007). Electroosmotic flow is directly proportional to the zeta potential and the electric strength applied to imply that with a low electric current,

electroosmotic flow is meant to be low and the opposite is true (Guedes et al., 2014).

2.8.7 PH distribution and its effects on the electrokinetic remediation process

The effective evaluation of the processes in an electrokinetic reactor requires the consideration of the behaviour of a particular system species in widely varying pH (Acar et al., 1995, Ouhadi et al., 2010). The movement of hydroxyl ions as a result of electroosmosis produces a pH variation along the matrix ranging from very acidic conditions at the sludge-electrode interface (usually the cathode) where the hydroxyl ions are deposed of, to very alkaline conditions at their source which is usually at the anode-electrode interface (Yang et al., 2005).

The increase in pH can enhance the separation of organic compounds in the matrix into negatively and positively charged organic compounds such as $RCOO^-$ radicals and H^+ which combine with other available species to form other complex compounds such as fatty acid salts. These substances could either enhance or inhibit the remediation process depending of their psychochemical properties (Yang et al., 2005). The inhibition of the remediation process due to the highly varying pH is at times avoided by the use of ammonia, citrate, oxalate, acetic acid or conditioning using ion exchange membrane (Acar et al., 1995, Shen et al., 2007, De Battisti and Ferro, 2007, Park et al., 2009).

In a similar manner, phase separation is also affected in that high pH in the cathode compartment leads to desorption of colloids from the liquid phase emulsions (Elektorowicz et al., 2006). A low pH which usually increases after some time in the system facilitates the release of contaminants of concern from the media (Acar et al., 1995, Rutigliano et al., 2008, Kim et al., 2009b, Zhang et al., 2014). In addition, acidic conditions in the system enhance electrochemical oxidation reactions in the system (Xu et al., 2014, Xu et al., 2017). The

convergence of the acid and alkaline front in the system affects the removal of the pollutant as a result focusing effects caused by precipitation of the pollutant at the pH junction (Li et al., 2012, Kimura et al., 2007a, Li et al., 2014, Rutigliano et al., 2008, Pazos et al., 2006). The problem of focusing effects can be resolved by controlling the pH through catholyte conditioning, use of chemical reagents or use of ion selective membrane (Li et al., 2012, De Battisti and Ferro, 2007). In some cases, the application of conditioning or processing fluids does not necessarily lead to the efficiency of the remediation process without pH control (Alcantara et al., 2010). In the removal of heavy metals, bioleaching can also overcome focusing effects by dissolving and disassociating the heavy metals into mobile ions (Xu et al., 2017).

Meticulous management of pH in an electrokinetic system is inexorable if the system is to have its greatest efficiencies in terms of contaminant removal (Yuan and Chiang, 2008). It should be noted that much as zeta potential is strongly dependant on the texture of the solid surface, it may also be affected by the pH of the pore solution, ionic strength and type of ionic species (Virkutytea et al., 2002, Xu et al., 2017). The relationship between zeta potential (ς) and pH was described by Park et al. (2009) as follows;

$$\varsigma(\mathrm{mV}) = 38.6 + 281e^{-0.48pH} \qquad (2.11)$$

2.8.8 Effect of surfactants application in the electrokinetic cell

Surfactants are mainly applied to increase the solubility and mobility of the contaminants in the electrokinetic system (Virkutytea et al., 2002, Reddy and Saichek, 2004). Due to the low hydrophobicity and solubility properties of organic contaminants, it is usually complex to remove them from a solid matrix unless a surfactant is applied to act as a flushing agent (Boulakradeche et al., 2015). The most dangerous organic pollutants are usually insoluble in water, non-ionic and have non ionisable molecules which make it impossible to remove these

contaminants with the two main processes of electromigration and electroosmosis (Cameselle et al., 2013). The removal of organic contaminants in this case requires simultaneous adequate electroosmotic flow and solubilisation of the contaminants which can only be achieved by the use of solvents and surfactants otherwise, electrokinetics has to be applied in combination with another method such as chemical oxidation and permeable reactive barriers (Cameselle et al., 2013). The solubilisation capacity of the surfactant is therefore very important for this to be achieved (Giannis et al., 2007). Through micellisation, surface tension reduction and solubilisation and increased adsorption, surfactants increase the rate of contaminant removal by altering the surface properties of the matrix leading to an enhanced electroosmotic flow (Gomes et al., 2012, Reddy and Shirani, 1996, Alcantara et al., 2010). When surfactants are applied, their hydrophilic heads allow them to manifest strong solubility properties in water while the hydrophobic tail will have more affinity for hydrophobic molecules such as organic molecules (Alcantara et al., 2010). Besides the cations and anions present in the system, surfactant effectiveness in the electrokinetic system strongly depends on the surfactant type/properties, solid matrix and other variable factors.

Amongst all the types of surfactants, neutral surfactants are the most preferred in electrokinetic remediation because they can easily be transported by electroosmosis as compared to cationic and zwitterionic surfactants which are highly toxic and highly interact with the negatively charged solid matrix leading to a slowdown of the remediation process (Boulakradeche et al., 2015, Reddy and Saichek, 2004). Anionic surfactants may inhibit the efficiency of the process because they tend to move towards the anode opposite to the electroosmotic flow (Boulakradeche et al., 2015, Reddy and Saichek, 2004). For example, the application of anionic surfactants enhances electrophoresis and electromigration by producing a negative zeta potential on the surface of the matrix while on the

contrary cationic surfactants inhibit the process by having strong interactions with the matrix (Gomes et al., 2012). Some non-ionic surfactants have tendencies of acquiring charges as a result of hydrogen bonding with some of its molecules or bonding with available cations whose effect could be the reduction of electroosmotic flow towards the cathode leading to a reduction in contaminant removal efficiencies (Reddy and Saichek, 2004). Nevertheless, anionic surfactants are more commonly used in the electrokinetic remediation process as compared to cationic surfactants because of their high solubilisation capacities and higher biodegradability potential in the environment (Alcantara et al., 2010). As far as use of surfactants in an electrokinetic system is concerned, researchers have in the previous years focused more on the use of cationic surfactants to remove anionic pollutants and the use of anionic surfactants to remove cationic pollutants. This means that removal of the pollutant greatly depends on binding of the pollutant with surfactant micelle which is as result of its valence (Yuan and Chiang, 2008). In the presence of surfactants in the pore fluid, electrophoresis is usually the dominant process (Reddy and Shirani, 1996). It was also reported that efficiency increases with increase in the duration of the experiment (Reddy and Shirani, 1996). Different results are therefore obtained depending on the type of the surfactant, the matrix, and the contaminant to be removed as shown in the table 2.4 below.

Table 2. 4: Application of surfactants in the removal of organic contaminants as adapted from Gomes et al. (2012).

Target contaminant	**Surfactant**	**Matrix**	**Removal efficiency %**
Chlorobenzene and trichloroethylene	Triton X-100, OS-20ALM	Spiked soil	85
Polycyclic hydrocarbons, BTEX such as benzene, toluene, ethylbenzene, xylenes	Cetyltrimethylammonium bromide	Spiked clay	97
Gasoil	Pannox 10, Citric acid	Spiked soil	87
Ethylbenzene	SDS	Spiked soil	98
Heavy metals and PAHs	Tween 80	Marine, contaminated and sediments	62–84 for metals and 18 for PAH
PAH (16 priority PAH)	(≥ 100 years) Tween 80	Contaminated soil	30
Lubricant oil and zinc	Tergitol	Contaminated soil	45
Phenanthrene	hydroxypropyl-beta-cyclodextrin	Spiked kaolin	75
Phenanthrene	Alkyl polyglycosides	Spiked kaolinite	98

Naphthalene and 2,4-dinitrotoluene	carboxymethyl-g-cyclodextrin	Spiked soil	83 and 89
Phenanthrene	Igepal CA-720	Spiked kaolin and sand	90
Phenanthrene	Alkyl polyglycosides	Spiked kaolin	75
Hexachlorobenzene	Tween 80 and bcyclodextrin	Spiked kaolin	80
DDT	Tween 80 and Sodium dodecylbenzenesulfonate (SDBS)	Spiked soil	13

Contrary to the positive results in the table above, Elektorowicz and Habibi (2005) investigated the effect of surfactants on the effectiveness of phase separation in an electrokinetic cell using an amphoteric surfactant (C12-C14-alkyl-dimethyl-betain) and concluded that the surfactant used did not improve the efficiency of the system. 63 % of water and 43 % of hydrocarbons was recovered without the use of a surfactant while 60 % of water and 50 % of hydrocarbon was recovered when the amphoteric surfactant was used indicating a reduction in water recovery but an increase in that of oil.

Elektorowicz et al. (2006) also concluded that the introduction of amphoteric surfactant to the system did not improve the total efficiency of the process. In contrary to this finding, Park et al. (2009) reported the increase in solubilisation of the lubricant oil from the soil into the pore fluid when a non-ionic surfactant was applied leading to increased removal of the hydrocarbons from the soil through the application of electrokinetics. When Giannis et al. (2007) applied sodium dodecyl sulphate in electrokinetic remediation of cadmium contaminated soil, they reported that SDS failed to effect the process because it was precipitated

as salt in the cathode compartment due to high pH contrary to when it was applied in the anode area where it remained stagnant due to its anionic nature.

The disadvantages related to the use of chemical surfactants, and other electrokinetic enhancement methods such as application of cyclodextrins, pH control and use of enhancement solutions are that their success depends on the type in case of surfactants, inclusion compounds between complexing agents and organochlorines if cyclodextrins are used. Regarding pH, the use of enhancement solutions depends on the enhancement solution used and there could be possible interferences between enhancement solutions and the addition of acids that poses environmental risks during pH control (Gomes et al., 2012).

Bacteria can produce biosurfactants which could be bio-demulsifiers (Rocha et al., 2017). Biodemulsifiers enhance demulsification by adsorbing to oil-water interfaces where they react with the existing emulsifiers resulting in the elimination of the thin film at these oil-water interfaces. This promotes coalescence of the dispersed droplets leading to two distinct continuous phases (Rocha et al., 2017). Similar to chemical surfactants, biosurfactants have been reported to promote, inhibit, or in other words have no effect on the remediation of the pollutant (Cameotra and Singh, 2008).

2.8.9 Effect of the electrokinetic process on microorganisms

In the process of performing electrokinetic remediation, very few studies have been done about the effect of the electrokinetic process on the survival, growth, movement and the enzyme activity of the microorganisms in the system (Kim et al., 2010, Lear et al., 2007, Wang et al., 2009). The bacteria in the soil are mostly affected by electroosmosis because it’s their transport mechanism when an electric field is applied (Kim et al., 2010, Schmidt et al., 2007). Mena et al. (2016) also strongly argues that microorganisms can be transported through the process of electrophoresis (Mena et al., 2016).

Unlike electroosmosis, pH, electrical potential and temperature variations can lead to the death of the microorganisms due to the halo-thermal environment that may not favour their survival (Kim et al., 2010, Lear et al., 2007). In their experiments to investigate the effect of electrokinetic remediation on indigenous microbial activity and community within diesel contaminated soil, Kim et al. (2010) claim that following the application of an electric field and changes in pH, the microbial cell count in the soil medium greatly reduced only leaving the halo-thermal bacteria *Bacillus* in existence with the highest cell count noted in the anode area (229 CFU g^{-1} soil) as compared to the cathode area (48 CFU g^{-1} soil). In the identification of the bacteria species within the system it was reported that *Bacillus* related strains were predominant in highly acidic conditions while *Pseudomonas and Rhodococcus* were abundant in the alkaline conditions of pH 5-8 (Kim et al., 2010). The same was reported by Lear et al. (2007) when applications of electrokinetics reduced bacteria and fungi counts by 17 % and 30 % respectively in the anode area. Microorganisms are most likely to move towards the cathode because of their surface charge (Mena et al., 2016). Peng et al. (2011) also reported that the combination of electrokinetics and bioleaching using *Thiobacillus* resulted into 78.6 % and 99.11 % removal of copper and zinc respectively but discovered that pH was an important factor of consideration for the bacteria to grow. In some cases, nutrients may also be applied to the system to enhance the bio-electrokinetic remediation process by promoting the co-metabolism of the contaminants (Schmidt et al., 2007, Kim et al., 2010).

The application of electrolytes may also, pose a detrimental effect on the microorganisms in the medium due to their acidity or alkalinity properties so their application should suitably be controlled to avoid the impact (Kim et al., 2010, Epelde et al., 2008). The hydrocarbon biodegradation efficiencies are highest in a more neutral pH as was observed by Kim et al. (2010) when they concluded that the degradation efficiencies of light hydrocarbons (C_{10}–C_{16}) were 23.2 % and

26.8 % under pH of 7 and 8 respectively whereas at extreme soil pH, the degradation efficiencies were 16.0 % and 18.9 % for pH 3 and pH 1 respectively. The explanation for the death or reduction of the microbial cell counts was because pH affected the bioavailability of the microbially required nutrients and also affected the cell membrane integrity and function (Kim et al., 2010). This, however, may not be reported regarding the electric field microbial impacts when a low Dc voltage is applied (Kim et al., 2010). The low electric field may actually increase bacterial growth, substrate utilisation and biodegradation in cases where an electric field does not exceed 10 mA with the enhancement from increased microbial oxygen uptake rate as a result of increased delivery to the microbial species in the medium due to electroosmosis (Kim et al., 2010, Atlas, 1981, Lear et al., 2007).

The conditioning agents and chelating agents added to the system are also most likely to have detrimental impacts on microorganisms due to their toxicity (Epelde et al., 2008). Some of the conditioning, complexing and chelating agents used include acetic acid, ethylenediaminetetraacetic acid (EDTA), citric acid and ethylene diamine disuccinate etc. (Zhang et al., 2014, Epelde et al., 2008, Yuan and Chiang, 2008, Jeon et al., 2015). Much as EDTA is the most commonly used due to its high chelating ability, other chelating agents such as citric acid and ethylene diamine disuccinate have been reported not to be as toxic as the former (Epelde et al., 2008).

2.8.10 Ex-situ application of the electrokinetic processing system

When an electrokinetic processing system is applied for remediation of contaminated soil, DC current is applied to the series of electrodes placed in electrode wells excavated in the contaminated soil. According to their charges, charged ions of different mediums (soil, the contaminant and water) migrate towards oppositely charged electrodes. Like lab scale findings, the pH drops at the anode because of the creation of the acid front at the anode chamber while the pH increases at the cathode because of a created base front at that chamber. Processing fluids such as acetic acids, humic or gallic acids are used to eliminate the pH imbalance at the electrodes and enhance solubilisation and ion migration (U.S.EPA, 1997, Yang et al., 2005).

CHAPTER THREE

METHODOLOGY

3.1 Materials

3.1.1 Chemicals and Reagents

Most of the chemicals including acetonitrile, ethyl acetate, hexane, acetone, and all the chemicals used in the preparation of the mineral salt medium where obtained from Merk, Germany. The mineral salt medium (MSM) sterilized by autoclaving at 121 ^{o}C for 15 min was used for the growth and production of biosurfactants. The medium was prepared as was reported by Trummler et al. (2003) by dissolving in 1 L of distilled water: 6.0 g $(NH4)_2SO_4$; 0.4 g $MgSO_4 \times 7H_2O$; 0.4 g $CaCl_2 \times 2H_2O$; 7.59 g $Na_2HPO_4 \times 2H_2O$; 4.43 g KH_2PO_4; and 2 mL of trace element solution. Plate count agar, nutrient agar and nutrient broth were prepared by dissolving the amounts indicated on the bottle in distilled water followed by autoclaving at 121 ^{o}C in order to sterilize for 15 min. The agar was poured on to the agar plates between 40-50 ^{o}C. The trace elements solution consisted of, 20.1 g L^{-1} EDTA (Disodium salt), 16 g L^{-1} $FeCl_3 \times 6H_2O$, 0.18 g L^{-1} $CoCl_2 \times 6H_2O$, 0.18 g L^{-1} $ZnSO_4 \times 7H_2O$, 0.16 g L^{-1} $CuSO_4 \times 5H_2O$ and 0.10 g L^{-1} $MnSO_4 \times H_2O$.

3.1.2 Petroleum contaminated soils

The soil used in these experiments composed of 71 % sand, 20 % silt and 9% clay obtained from Pretoria, South Africa. The soil had initial total organic carbon content of 4.03 % and particles sizes of 74.13% > 425 µm, 21.45 % between 425-300 µm and 4.42 % < 300 µm. Initially, the soil was sieved using a 2 mm sieve to remove large coarse materials such as leaves and stones. The soil was sterilized then spiked with engine oil obtained from a tribology laboratory at the University

of Pretoria to achieve 150 mL/kg of soil contamination after homogenous mixing using an overhead stirrer and kept for 14 days before the experiments.

3.1.3 Microbial culture, media and growth conditions

Strain PA1 was obtained from API tank sludge in South Africa by Selective enrichment to obtain efficient hydrocarbon degraders in 250 mL Erlenmeyer flasks according to Trummler et al. (2003). The enrichment was done by inoculating 5 gm of sludge into 100 mL of MSM enhanced with 5 % (v/v) sunflower oil as a carbon source and energy source at 30 ^{o}C and 120 rpm for 7 days. A total of 6 subsequent enrichments was done to isolate the PHC degrading consortium by transferring 10 mL of enriched culture into another flask containing 100 mL of freshly sterilized MSM with 5 % (v/v) sunflower oil and incubated. The isolation and purification of the consortium was done by spreading and streaking on Luria-Bertani agar. 100 µL of culture dilutions were spread on the LB agar plates and incubated at 37 ^{o}C for 72 hours. The isolates were later subjected to 16S rRNA gene sequencing analysis basing on colony morphology. Considering that in previous studies pseudomonads have been reported to have great hydrocarbon-degrading and biosurfactant producing capabilities (Das and Chandran, 2011), it was upon the researcher that if such a strain existed amongst those isolated, then that strain be used ahead of others. Strain PA1 was selected for use in this research because it was a pseudomonad but also due to its effective biosurfactant production and hydrocarbon degrading capabilities.

3.1.4 Screening for biosurfactant production

The isolated cultures were screened for biosurfactant production using the drop collapse method and the oil spreading test. In the drop collapse method, 2 µL of mineral oil was added to each well of a 96-well microtiter plate. The plate was equilibrated for 1 h at room temperature, and then 5 µl of the culture was added

to the surface of oil (Bodour and Miller-Maier, 1998). The shape of the drop on the surface of oil was inspected after 1 min. The result was negative If the drop remained beaded while the result was positive If the drop collapsed. Cultures were tested in triplicate. The Oil spreading test was also done as described by Morikawa et al. (2000) in which 50 mL of distilled water was added to a large petri dish (25 cm diameter) followed by the addition of 20 µl of oil to the surface of the water. 10 µl of culture were then added to the surface of the oil. The diameter of the clear zone on the oil surface was measured and related to the concentration of biosurfactant. Mineral salt medium and distilled water without cells were used as controls for both screening tests.

3.1.5 Characterization and identification of microbial species

Pure cultures of biosurfactant producing isolates were characterized using the 16S rRNA genotype fingerprinting method. This was achieved by extracting the DNA from the pure cultures according to the protocol described in the Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA). The 16S rDNA region was amplified by PCR using the primers 8F (50-GGATCCAGACTTTGATYMTGGCTCAG-30) and 907R (50-CCGTCAA TTCMTTTGAGTTT-30). The amplified genome was purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). Amplifications were performed in a GeneAmp PCR System 9700-Thermocycler from PE Applied Biosystems. PCR products were analysed together with a molecular weight ladder. The DNA sequence for each pure colony was then uploaded to the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). A phylogenetic tree was constructed from the identified 16S rRNA sequences using the neighbour-joining method in the MEGA Version 6 software. The sequencing was done at the Department of Microbiology of the University of Pretoria.

3.1.6 Biosurfactant production, recovery and purification

To produce biosurfactant, a pure strain of PA1 was inoculated in Erlenmeyer flasks containing 200 mL of sterilised nutrient broth in a sterile environment. The flask was then incubated at 35 ºC, pH = 7 and 250 rpm for 48 hours. The cells were harvested by centrifugation at 10,000 rpm at 4 ºC for 10 minutes. The cells were then transferred to larger erlenmeyer flasks containing 1,000 mL of MSM supplemented with 3 % oil (v/v) and incubated at 35 ºC, pH = 7 and 250 rpm. The harvest was made every after 7 days and the process repeated for a period of 8 weeks. To obtain a cell free supernatant after the harvest for eight weeks, the pH was adjusted to 7, cells were then removed by centrifugation (12,000 rpm at 4 ºC for 20 min). Crude biosurfactant was precipitated from the supernatant by adding 6 N HCl to a pH of 2.0. The acid precipitate was recovered by centrifugation (12,000 rpm at 4 ºC for 20 min). The biosurfactant was further extracted with chloroform and methanol (2:1) followed by evaporation of the solvent in a vacuum. The obtained residue was dissolved in methanol and filtered through a 0.22 mm filter (Millipore). The crude extracts were purified through a Silica gel column (silica gel 60 (63–200 mesh); Merck KGaA). The impurities were further removed from the extract by eluting with chloroform, and twice using chloroform and methanol in 80:20 v/v (100 mL), then 35:65 v/v (100 mL) respectively. The solvents were finally evaporated from the eluted extract 40 ºC.

3.1.7 Biosurfactant characterization

3.1.7.1 Thin Layer Chromatography (TLC)

10 mg of the biosurfactant extract dissolved in methanol was applied near the bottom edge of the TLC plates in small spots to have revelations of the chromatographs. Biosurfactants were characterized by thin layer chromatography (TLC) on silica gel 60 plates (F254; Merck). The plates were developed with chloroform: methanol: water (65:15:4, v/v) as the solvent system. Spots were

revealed by spraying with 0.35 % (w/v, in acetone) ninhydrin for detection of compounds with free amino groups. After spraying with ninhydrin, the plates were heated at 110 ^{o}C for 5 min until the appearance of the respective colours (Noparat et al., 2014).

3.1.7.2 Fourier transform infrared spectroscopy (FTIR)

To identify the chemical bonds and the functional groups present in the chemical structures the Perkin Elmer 1600 Fourier Transform Infra-Red (FTIR) spectroscopy equipped with an Attenuated Total Reflectance (ATR) Crystal Accessory (Perkin Elmer, Connecticut, USA) was used. The sample was prepared by mixing 1 mg of crude biosurfactant with 100 mg of KBr and pressed with a load for 30 s to obtain translucent pellets. The infrared scan was performed over 400-4000 cm^{-1} with a resolution of 2 cm. The reflectance spectra were recorded and averaged over 32 scans, using the total internal reflectance configuration with a Harrick™ MVP-PRO cell consisting of a diamond crystal. Spectra were viewed and analyzed by Spectrum 10™ Software (Perkin Elmer) (Bezza and Chirwa, 2015).

3.1.7.3 Determination of surface tension and critical micelle dilution (CMD)

The surface tension of the biosurfactant supernatant was determined using the Krüss Tensiometer (K11 model – Germany) equipped with a 1.9 cm platinum ring based on the Du Nouy ring method (Rodrigues et al., 2006). The measurements were done at room temperature in triplicates to present values as derivatives of averages of independent measurements. Surface tensions of diluted biosurfactants (10 different dilutions) were determined to express the concentrations of the biosurfactants in critical micelle dilution. The dilutions were made using a phosphate buffer solution (10 mM KH_2PO_4/K_2HPO_4 and 150 mM NaCl with pH adjusted to 7.0) followed by surface tension measurements as described above.

3.1.7.4 Evaluation of the demulsification potential of the biosurfactants.

W/O model emulsions were prepared according to the following protocol (Coutinho et al., 2013). The mode emulsions (W/O) were prepared by mixing 0.2 % Tween 80 and either kerosene, hexane or toluene at 1:2 (v/v) at 24,000 rpm for 2 min in a Turrax-type agitator (Marconi) to produce three different emulsions. O/W emulsions of Tween-Triton-water-kerosene were obtained by producing a stock solution of kerosene prepared by mixing 2.5 mL of triton with 100 mL of kerosene on a stir plate, and a stock solution of Tween-80–water was prepared by adding 200 µL of tween in 100 mL de-ionized water. Tween-Triton-water-kerosene emulsion was finally obtained by mixing 0.9 mL Tween-80–water and 2.1 mL triton-kerosene solutions at 24,000 rpm for 2 min. The emulsion type was then verified by the Oil Red O-test (Coutinho et al., 2013).

Demulsifcation evaluations were done by adding 300 µL of biosurfactant supernatant in tubes of known dimensions containing 2 mL of emulsion and sealed. This was followed by agitation in a vortex for 2 min at 13,800 rpm to achieve complete mixing. The tubes were kept undisturbed in an upright position in a water bath at 35 ^{o}C. The changes in the volume of the oil phase (top), water phase (bottom), and emulsion phase (in-between) were recorded at 24-hour time intervals for up to 5 days. Demulsification of emulsions was based on the volume of separated emulsion compared to the original volume (EV) and expressed as the demulsification percentage (Coutinho et al., 2013) – % Demulsification = [(initial volume (2 mL) - final emulsion volume at interphase (mL))/initial volume (mL) 100]. All experiments were carried out in triplicates.

3.2 Scanning Electron Microscopy (SEM) for analysis of colloids in the anode compartment resulting from electrophoresis

The size and compaction of the colloid particles was carried out by means of a scanning electron microscope Vega 5135 MM from Tescan company (SE Detector, 30 kV, high vacuum 5×10^{-3} Pa) working with EDX Link 300 ISIS from Oxford Instruments (Detector Si (Li), 30 kV, low vacuum 10 Pa, resolution 60 eV). The coagulated particles in the anode compartment were meticulously collected and dried at 115 ^{o}C. The samples were prepared by adding 25 % Glutaraldehyde: Formaldehyde (1:1, v/v in 3 ml of distilled water and 5 ml of dilute 1.5 M phosphate buffer) to fix for one hour. The sample was washed with the phosphate buffer 3 times for 15 minutes after the removal of the fixative solution. The buffer was removed to add 1 % osmium tetraoxide solution and fixed again for one hour in a fume cupboard. The fixative solution was removed to wash three times with phosphate buffer. The buffer was removed to dehydrate the sample by grade series of ethanol (30 %, 50 %, 70 %, 90 % and 3 ×100 %) for 15 minutes each. The sample was left in 100 % ethanol for 30 minutes. The ethanol was removed to add a 50: 50 v/v mixture of hexamethyldisilazane (HMDS) and 100 % ethanol for one hour while covered. The HMDS was removed to add fresh HMDS and left for open for the sample to dry. The samples were mounted and coated with gold to examine with the microscope.

3.3 Electrokinetic set up

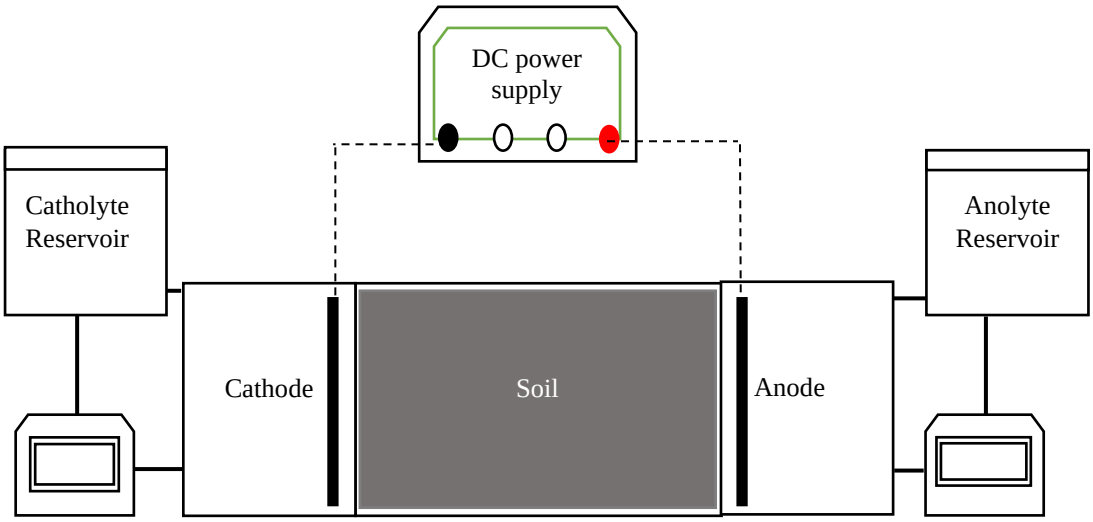


Figure 3. 1. Schematic view of the electrokinetic reactor

The electrokinetic reactor was meticulously constructed from acrylic glass to make 3 compartments; a soil compartment (160.5 mm ×150 mm ×150 mm) and two electrode compartments (150 mm × 90 mm × 150 mm) so that one of them constituted the anode and the other one the cathode with outlets to electrolyte overflow reservoirs. Graphite electrodes (100 mm long × 20 mm diameter) were located into the electrode compartments at specified distances apart and connected to the DC power supply (0-30 V,0-3 RS-IPS 303A) using stranded iron wires. Distilled water was used as the electrolyte with the electrode-medium compartment interfaces fixed with glass filters (Whatman microfiber Grade GF/A: 1.6 μm) to allow electroosmotic flow across the cell.

3.4 Electrokinetic experiments

3.4.1 Experiments to determine the effect of voltage

4 experiments were carried out under the conditions shown in the table 3.1. 2000 g of soil spiked with oil was treated for all the experimental conditions described in triplicates.

Table 3. 1: Experimental conditions

Experiment	Microbial Conditions	Voltage (V)	Distance between electrodes	Time to run experiment in hours
A	22 g/L of biosurfactant + 30 g of cells	30	185 mm	240
B	22 g/L of biosurfactant + 30 g of cells	10	185 mm	240
C	30 g of bacterial cells	30	185 mm	240
D	30 g of bacterial cells	10	185 mm	240
Control 1	No biosurfactants and cells	30	185 mm	240
Control 2	No biosurfactants and cells	10	185 mm	240

3.4.2 Experiments to determine the effect of the distance between electrodes

Graphite electrodes (100 mm long × 20 mm diameter) where located into the electrode compartments for the first three distinct experiments at specified fixed electrode spacings of 185 mm, 260 mm and 335 mm while the fourth involved continuous movement of electrodes to have spacings of 335 mm, 260 mm and 185 mm as a continuous approaching electrode configuration with all the electrodes connected to the DC power supply of 30 V. The first experimental set ups were individually carried out for the three electrode spacings for 10 days each with 2 kg of contaminated soil inoculated with 30 g of cells and 200 mL of cell free supernatant with 22 g/L of biosurfactant. In the second set up electrode distances were reduced every 3 days from 335 mm, 260 mm to 185 mm with 2 kg of contaminated soil inoculated with 30 g of cells and 200 mL of cell free

biosurfactant supernatant with 22 g/L of biosurfactant. All the experiments were run in triplicates.

3.4.3 Experiments to determine the effect of variation in biosurfactant concentrations

2 kg of soil spiked with oil were mixed using an overhead stirrer with 30 g of bacterial cells produced for 24 hours and with either 26 g/L, 56 g/L or 84 g/L of biosurfactants to have three distinct experiments based on different biosurfactants concentration amendments.

In all experiments the medium compartment was divided into seven sections normalized to the nearest cathode to allow measurements of pH, bacterial counts and total organic carbon. Electroosmotic flow, pH, current measurements and bacterial counts were made every after 24 hours. To determine the number of viable cells, 10 mL of an aliquot were picked from each of the seven sections in the soil compartment at 10 mm, 30 mm, 50 mm, 70 mm, 100 mm, 130 mm and 160 mm normalized distances from the cathode including samples from the anode and cathode compartments every after 48 hours to determine colony forming units (CFU) at each section as formally described by (APHA, 2005).

3.5 Total Carbon analysis

Solid samples were picked from each of the seven sections in the soil compartment at 10 mm, 30 mm, 50 mm, 70 mm, 100 mm, 130 mm and 160 mm normalized distances from the cathode after 240 hours. The samples were air dried for 5 days and grinded to the smallest particles using a mortar and pestle. The fine samples were ready for analysis in the Schimadzu Total Organic Carbon Analyzer after they were sieved to remain with particles small enough to go through a 600 µm mesh. The solid sample boats were decontaminated of carbon residue by brush washing under flowing tap water followed by rinsing with distilled water. The boats were then soaked in 2 M hydrochloric acid for 10

minutes and heated in a furnace at 900 ^{o}C for 10 minutes and left to cool before running a sample.

3.6 Oil recovery

Oil recovered after the electrokinetic process of remediation was considered as the oil that was displaced from the soil bed to settle on top of the water layer in the three reactor compartments. To make the measurement the thickness of the oil on top of the water was measured in each of the compartments and multiplied by the known measurements (oil thickness/height x length of the compartment x width of the compartment) of the reactor to obtain the volume as was done by other researchers (Dastgheib et al., 2008, Bezza and Chirwa, 2017).

CHAPTER FOUR

CULTURE ISOLATION AND BIOSURFACTANT PRODUCTION

4.1 Microbiological Studies

4.1.1 Screening and identification of biosurfactant producing bacteria

Oil sludge was used as the source for the microbes. Much as it could be toxic for bacteria to survive in oil sludge, it is effective to take samples from areas where organisms could be slightly protected such as in sediments or flocs of solid matrices. In such environments, concentration gradients could be created due to mass transport resistance which could result in the creation of safe havens around or inside solid matrix granules for some species of bacteria to survive. Indeed, a total of three different strains were isolated from the oil sludge sample. The hydrocarbon-degrading and biosurfactant producing strain PA1 obtained after isolation and passing the biosurfactant screening test using the drop collapse method and the oil spreading test was chosen for use in these studies. The strain was chosen on the basis of morphology and colour on agar plates to strategically choose the strain that showed the physical properties of a pseudomonas species. This is because pseudomonas species have been reported to have the greatest capability of degrading hydrocarbons and producing biosurfactants (Das and Chandran, 2011). The strain was identified using the 16S rRNA sequence analysis. The 16S rRNA sequence of PA1 showed the highest similarity to *Pseudomonas aeruginosa* with query cover of 100 % as shown in the phylogenetic tree presented in Figure 4.1 below.

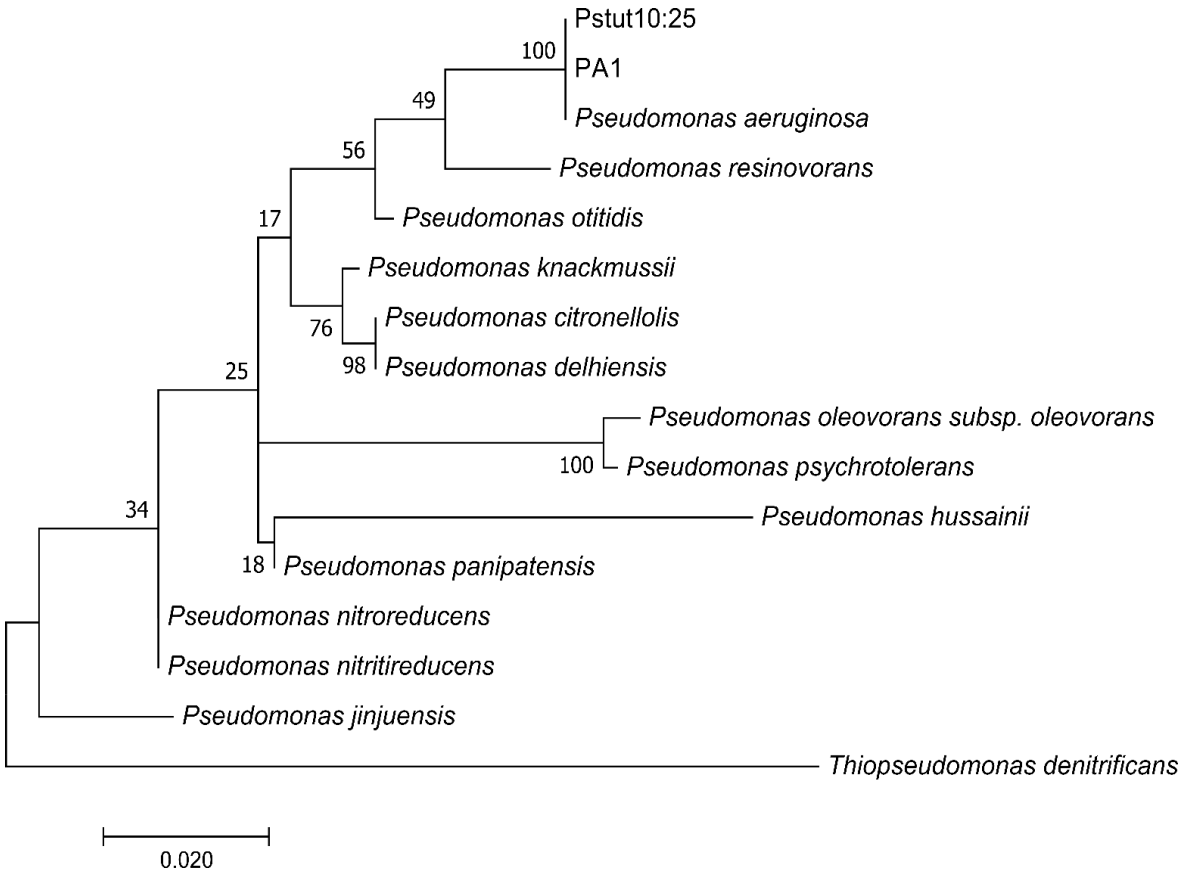


Figure 4. 1. Phylogenetic tree based on the 16S rRNA genotype fingerprinting method with a scale bar corresponding to 0.020 estimated nucleotide distance per sequence position.

4.1.2 Characterization of biosurfactants

4.1.2.1 *Biosurfactant yield, surface tension, and critical micelle concentration of the produced biosurfactant.*

The yield of biosurfactants during production depends on the culture, carbon source, medium, and the environmental conditions. The yield of Pseudomonas aeruginosa PA1 used in the studies after production was 140 g/l after extraction. Biosurfactants as surface active substances highly depend on their ability to reduce surface and interfacial tension. The biosurfactants produced reduced the surface tension of water from 71 mN/m to 30.35 mN/m and had a *cmc* value of

156 mg/L (Figure 4.2) relating to the results reported by other researchers (Câmara et al., 2019).

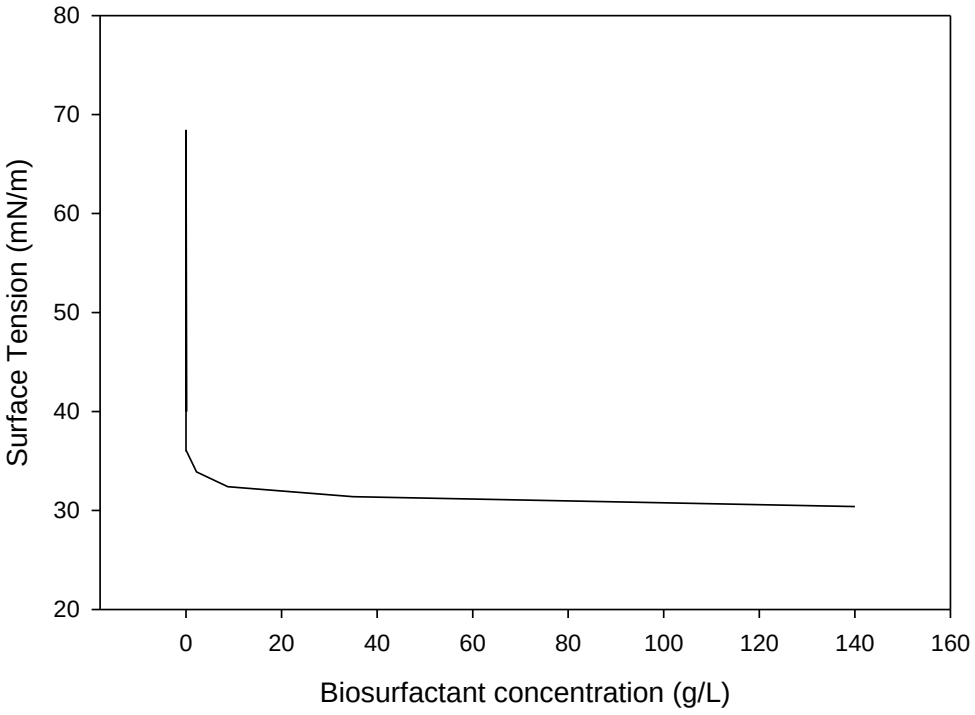


Figure 4. 2. Surface tension versus concentration of biosurfactants produced by *Pseudomonas strain* PA1

4.1.2.2 FTIR characterization

The FTIR was used to study the chemical functional groups of the biosurfactants produced by the PA1 strain with fingerprint areas between 4000 and 400 cm^{-1}. The results showed a high similarity with a typical spectrum of rhamnolipids with the vibrations showing presence of peptides and aliphatic hydrocarbons (Fadhile Almansoory et al., 2017, Rikalovic et al., 2012). The infrared spectrum in Figure 4.3 shows the FTIR analysis. The absorption bands at 3336 and 889 cm^{-1} represents O-H symmetric stretching, deformations at 2920, 2849, 726 cm^{-1} are for C-H due to compositions of sugar residues, symmetric stretching of C=O of the carboxylate group occur at 1737 and 1380 cm^{-1}, and C-H/O-H deformations reoccur at 1153, 1118, and 1670 cm^{-1} (Rikalovic et al., 2012). Additionally, the

bands at 1459 cm^{-1} represent C-O stretching while peaks at 975 cm^{-1} represent O-H deformations (Fadhile Almansoory et al., 2017).

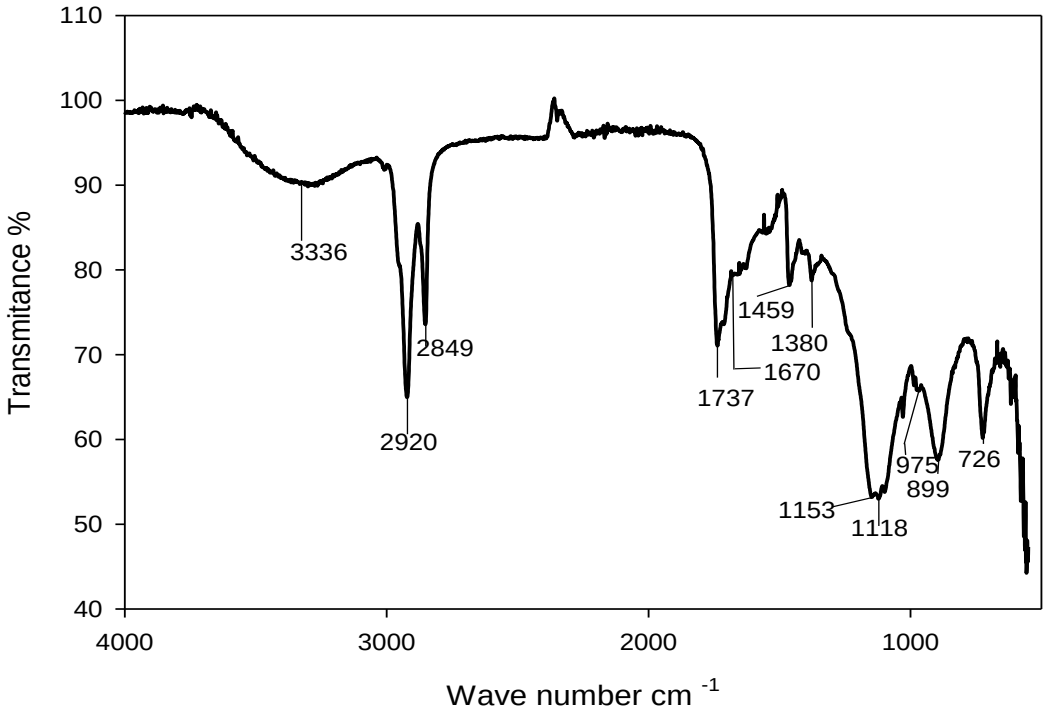


Figure 4. 3. Fourier-transform infrared spectra of the biosurfactant produced by the strain PA1

4.1.2.3 Thin-layer chromatography (TLC) analysis

The TLC results of the biosurfactant extracted from the acid precipitate revealed a pink spot on the plates with an Rf value of 0.42 when sprayed with ninhydrin as shown in Figure 4.4 signifying the presence of amino acids in the biosurfactants as similarly reported in other studies (Sriram et al., 2011). The low Rf also shows the polar property of the biosurfactant made up of a mono-rhamnolipid (George and Jayachandran, 2013).

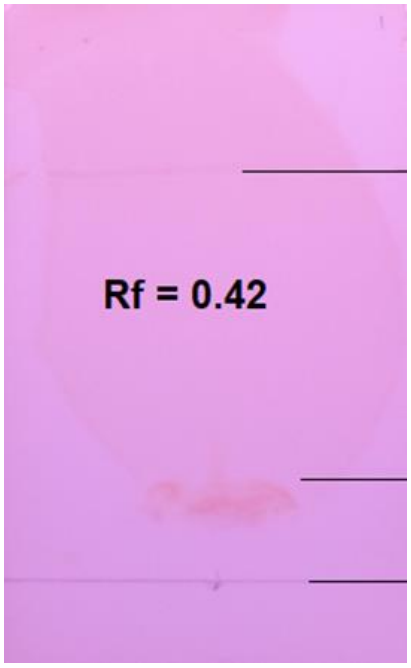


Figure 4. 4. Analysis of Thin Layer Chromatography of the biosurfactant produced by the strain PA1 revealing a pink pigment after spraying with ninhydrin to produce an Rf value of 0.42

4.2 Evaluation of the demulsification potential of the biosurfactants in O/W and W/O emulsions

The n-Hexane W/O emulsion produced the highest demulsification of 85.7 % as compared to toluene with 59.3 % and Kerosene with 55.6 % in five days (Figure 4.5 and 4.6). The O/W emulsion of Tween-Triton-Kerosene produced the lowest demulsification of 35.3 % (Figure 4.5 and 4.7). Emulsions with a continuous phase of water (W/O emulsions) were easier to break as compared to those with a continuous phase of kerosene (O/W emulsions). This can be attributed to the viscosity of the emulsions with emulsions of water being less viscous enabling the breakup of the emulsion as compared to the more viscous emulsions with oil (Coutinho et al., 2013). The findings also indicate that the strain produced demulsifiers with the ability to easily break emulsions with only one type of organic phase as compared to those with multiple organic phases which is an agreement with some previous reports where the biosurfactants produced by their

pseudomonas strains failed to efficiently demulsify both types of emulsions (Coutinho et al., 2013, Huang et al., 2009). The ability of the surface-active compounds produced by *Pseudomonas aeruginosa* strain PA1 to break emulsions shows that the surface-active agent produced is a biosurfactant with the ability to emulsify immiscible liquids and demulsify or break emulsions as compared to bioemulsifiers (that were not required in these studies) that only have the capacity to emulsify (Uzoigwe et al., 2015).

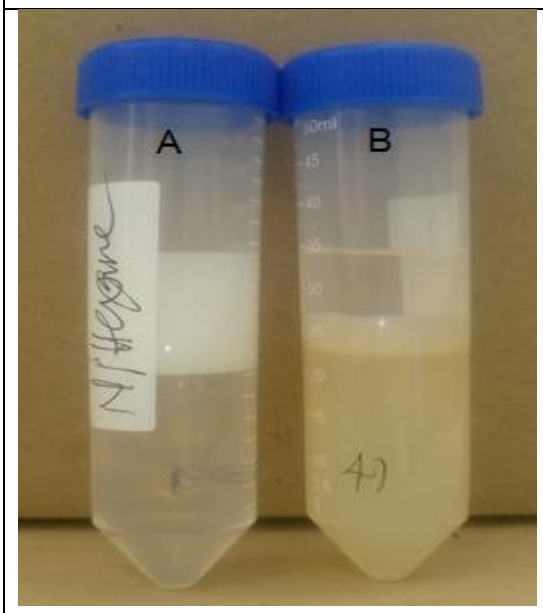
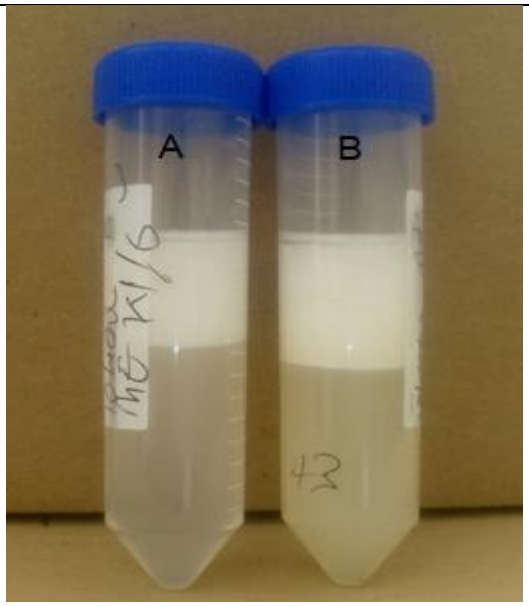
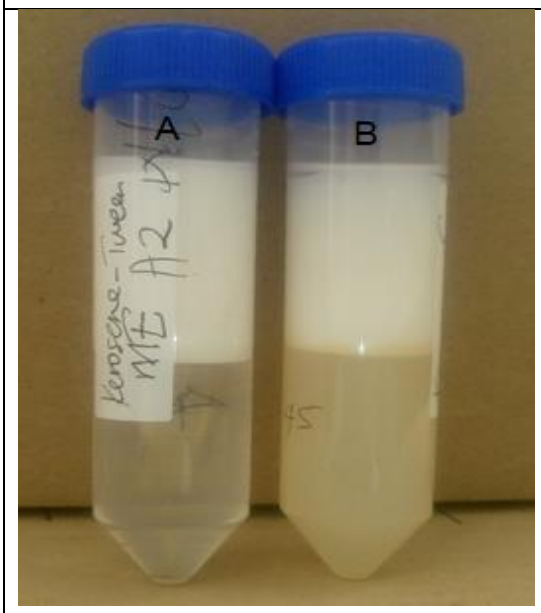
A: Control: Emulsion + water **B: Emulsion + Biosurfactant**	
	
W/O emulsions of n-Hexane	W/O emulsions of Toluene
	
W/O emulsions of Kerosene	O/W emulsions of Tween-Triton-Kerosene

Figure 4. 5. Demulsification of O/W and W/O emulsions by the biosurfactant supernatant

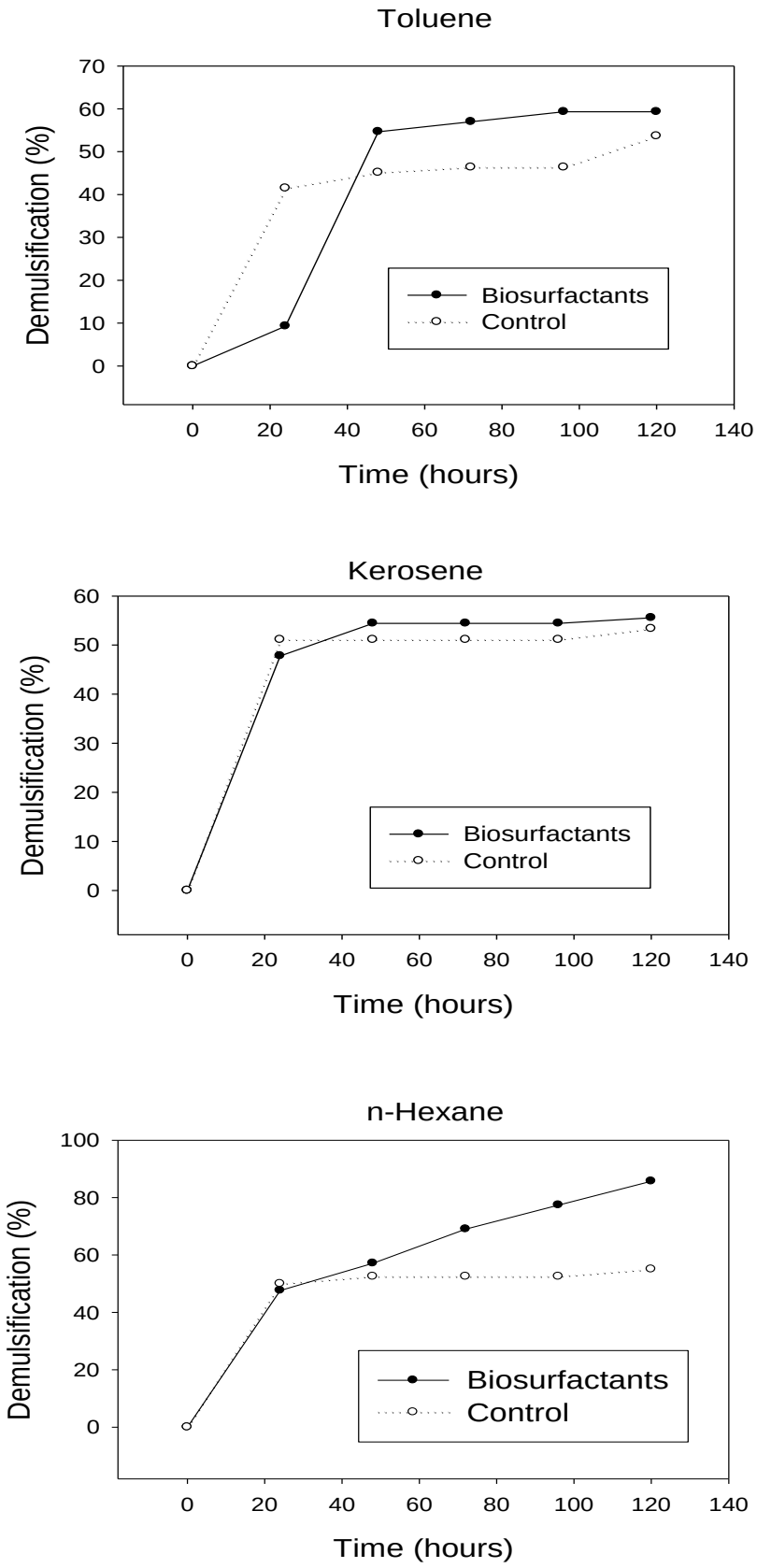


Figure 4. 6. Demulsification effect of the biosurfactant supernatant on W/O emulsions of Toluene, Kerosene and n-Hexane

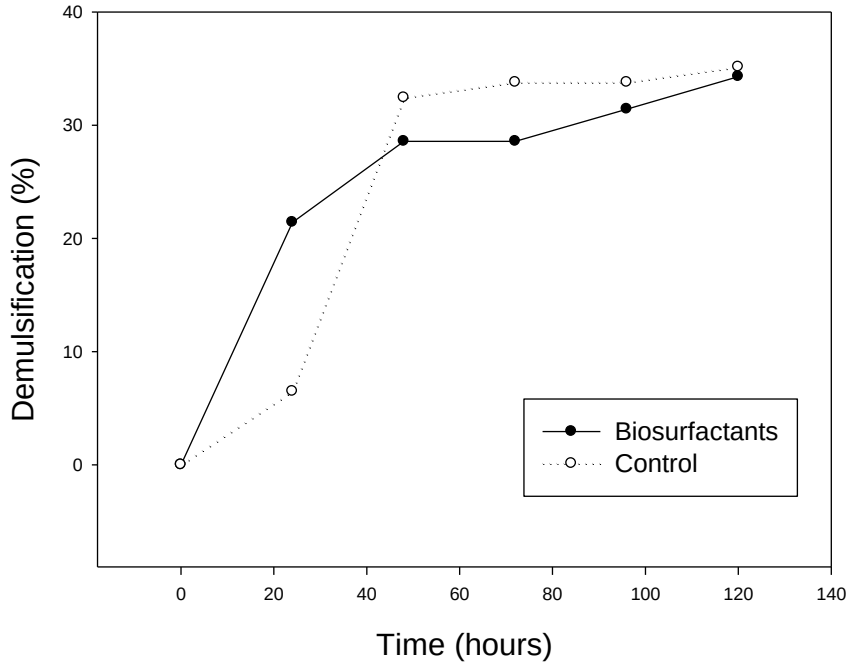


Figure 4. 7. Demulsification effect of the biosurfactant supernatant on O/W emulsions of Tween-Triton-Kerosene

Conclusion

The biosurfactant produced by strain PA1 shows great potential in the enhancing the demulsification of stable emulsions.

CHAPTER FIVE

BIOELECTROKINETIC REMEDIATION WITH VARRIATION IN VOLTAGE

5.1 The effect of voltage on the electrokinetic remediation of petroleum contaminated soil

Four experiments were carried out under the conditions shown in the Table 5.1. 2 kg of soil spiked with oil were treated in all the experiments for ten days. These studies were meant to evaluate the effect of voltage and biosurfactant application on the remediation process.

Table 5. 1: Experimental conditions

Experiment	Microbial Conditions	Voltage (V)	Distance between electrodes	Time to run experiment in hours
A	22 g/L of biosurfactant + 30 g of cells	30	185 mm	240
B	22 g/L of biosurfactant + 30 g of cells	10	185 mm	240
C	30 g of bacterial cells	30	185 mm	240
D	30 g of bacterial cells	10	185 mm	240
Control 1	No biosurfactants and cells	30	185 mm	240
Control 2	No biosurfactants and cells	10	185 mm	240

5.1.1 Oil Recovery

Oil was observed to coalesce vertically in the soil compartment as it oozed out of the solid matrix in all experiments (Figure 5.1.B). The highest oil recovery comparing experiments run under different voltages was observed in experiment A and B both of which were under application of biosurfactants in the first 96 hours as compared to the controls and those in which only biosurfactant free cells were inoculated as seen in Table 5.1 and 5.2. Most of the oil recovered remained in the soil compartment after 96 hours with miniature amounts moving as part of the electroosmotic flow to both the anode and cathode compartments (Table 5.1, Figure 5.1.C and Figure 5.1.D). The volume of the electrolyte increased in the cathode compartment as it reduced in the anode compartment indicating that the electroosmotic flow was towards the cathode with dominance of water and very low oil volume (Figure 5.1). With the surface charge of soil being predominantly negative, the electroosmotic flow is expected to flow towards the cathode (Yang et al., 2005). The net negative charge on the soil surface is as a result of both the variable and permanent charge emanating from the ionisable hydrogen ions and isomorphous substitution respectively. The variable charge is therefore dependant on solution pH since it varies depending on the sorption and desorption of H^+ and OH^- ions on the soil surfaces from the pore fluid (Park et al., 2009). With oxidation and reduction reactions happening in the electrode compartments, there is a formation of the acid front at the anode and an alkaline front at the cathode on immediate application of an electric field but as ions start migrating, the pH dynamically changes across the system as the H^+ ions move towards the cathode; This explains why the electroosmotic flow towards the cathode reduced as the acid front moved further away from the anode area towards the cathode with a possibility of reversed electroosmosis (from cathode towards the anode) due to the change in the soil surface charge influenced by reduction in pore fluid pH. The experiments were however stopped before the acid front covered more

than 15 mm from the anode compartment to observe a significant increase in the volume of the anolyte as a result of reversed EOF.

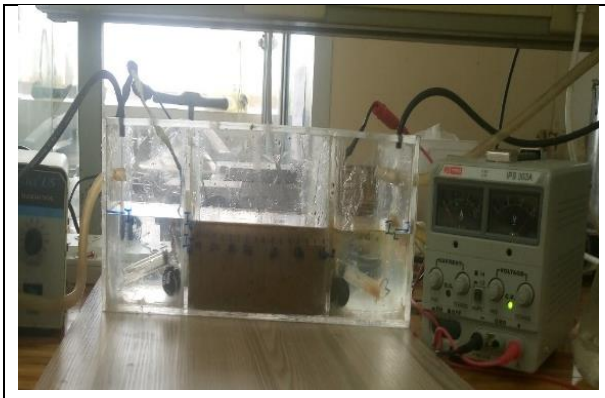
Table 5. 2: Volume of oil recovered in the soil compartment and that transferred to the electrode compartments due to electroosmotic flow in 96 hours

Experiment	**Oil recovered in different compartments**			**Total Oil Recovery (%)**
	Anode (mm^3)	**Soil Compartment (mm^3)**	**Cathode (mm^3)**	
A (biosurfactants +cells)	54,000	144,450	27,000	75.15
B (biosurfactants +cells)	13,500	96,300	13,500	41.10
C (only cells)	27,000	96,300	27,000	50.10
D (only cells)	27,000	72,225	13,500	37.58
Control 1 (30 V)	33750	96,300	27000	52.35
Control 2 (10 V)	36112.5	60187.5	20,250	38.85

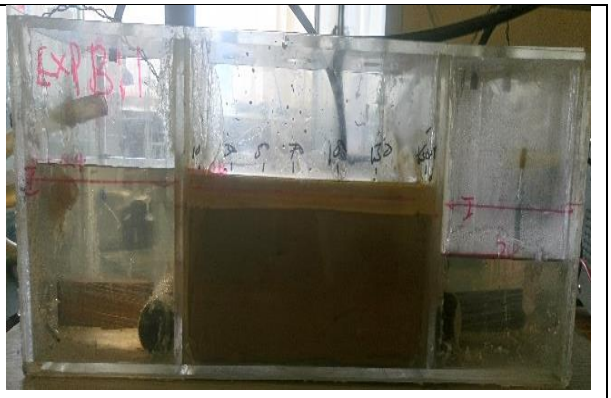
According to Helmholz–Smoluchowski theory described in equation 5.1 below, it can be concluded that the higher the viscosity of the liquid the lower the electroosmotic flow; With viscosity of water being generally lower than that of oil, it can explain why the electroosmotic flow was more dominated by water as compared to oil leading to horizontal stagnation of most of the recovered oil in the soil compartment instead of moving into the electrode wells. This is also in agreement with Yang et al. (2005) who argues that the process of electroosmosis can be affected by viscosity and the molecular size of the water or oil. The larger the size of the molecules the lower the electroosmotic rate since the liquid phases may not easily go through the filter to the electrode chambers. This can affect the rate of oil recovery as opposed to dewatering as oil has larger molecules which means it’s out competed by water which has smaller ones. The difference in the

oil recovered in the first 96 hours can only be explained by the activity of the biosurfactant which demulsified the contaminated soil leading to more oil recovered as compared to the control and when only cells were inoculated into the reactor. This means that the application of biosurfactants to the system produces the combined effect of electro-demulsification and demulsification by the biosurfactants leading to higher oil recovered. The oil recovered in the controls and experiment C and D was basically because of electro-demulsification since the two experiments (C and D) failed the biosurfactant test in the first 144 hours. Experiment C and control 1 produced very similar results while experiment D and control 2 also produced very similar results. This is because cells in experiment C and D had unsubstantial effects on the experiments since there was no production of biosurfactants by the microbes. The anolyte in the system became more turbid with time due to the movement of colloids towards the anode well; a process known as electrophoresis (Figure 5.1.C). These coagulated and sedimented in the compartment forming a very observable yet so distinct difference between the anolyte and the catholyte since the catholyte was quite clear. Electrophoresis in this case also enabled the bacteria to move from one the medium compartment to the anode compartment.

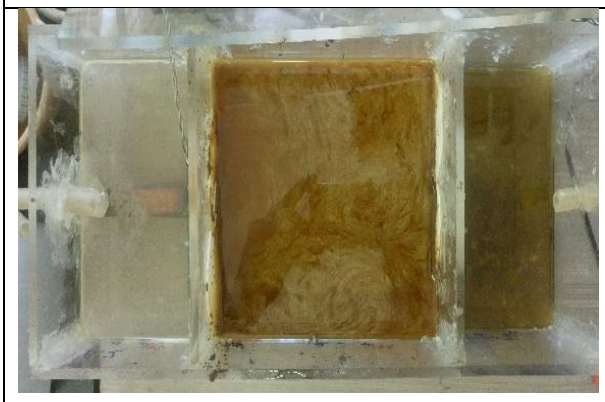
$$EOF = \frac{-D\varepsilon_0 Z}{\mu} E_x \qquad (5.1)$$



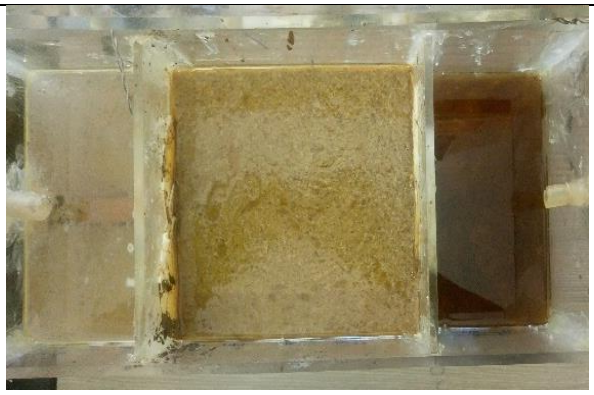
A



B



C



D

Figure 5.1. Figure 5. 1. A-Set up at the beginning of the experiment; B-High electroosmotic flow from the anode to the cathode and Oil coalescence in the medium chamber after 24 hours; C-High oil stagnation at the cathode-medium interface; D-High oil flow to the anode compartment

5.1.2 Current

The highest applied voltage of 30 V produced the highest current of 2.44 mA as compared to the lower electric potential of 10 V which produced 0.79 mA. Figure 5.2 shows that the highest current values were registered at the beginning of the experiment and started diminishing with time. The high current values observed during the initial stages of the process were due to the high electromigration of ions in the system which continues until equilibrium is reached due to reactions between the ions and the compounds in the system (Pham et al., 2009). In most electrokinetic reactors, electric current increases quickly during the first few

hours and then gradually thereafter. This is due to resistance in the interface between electrodes and the electrolyte which increases because of concentration polarization and water dissociation and because ions with positive or negative charges move to the two ends of the electric cell as a consequence of electrodialysis, which results in the drop of ionic strength in soils and the current (Wang et al., 2007). Comparing experiments run with similar voltages, experiment C and D produced higher currents the than the controls and experiment A and B respectively but they did not produce higher oil recovery than the later signifying that the biosurfactant had produced a significant baseline recovery that couldn't be overrun by a small change in current. In the same vain the electroosmotic flow was highest during the beginning of the experiment and reduced with a reduction in current. Considering Helmholz–Smoluchowski theory represented by equation (5.1) to include electric field (Ex), electroosmotic flow (EOF in m/s), dielectric constant (D), vacuum permittivity (ε_0), and fluid viscosity (l), It is in agreement with the results since electroosmotic flow is directly proportional to the electric field applied.

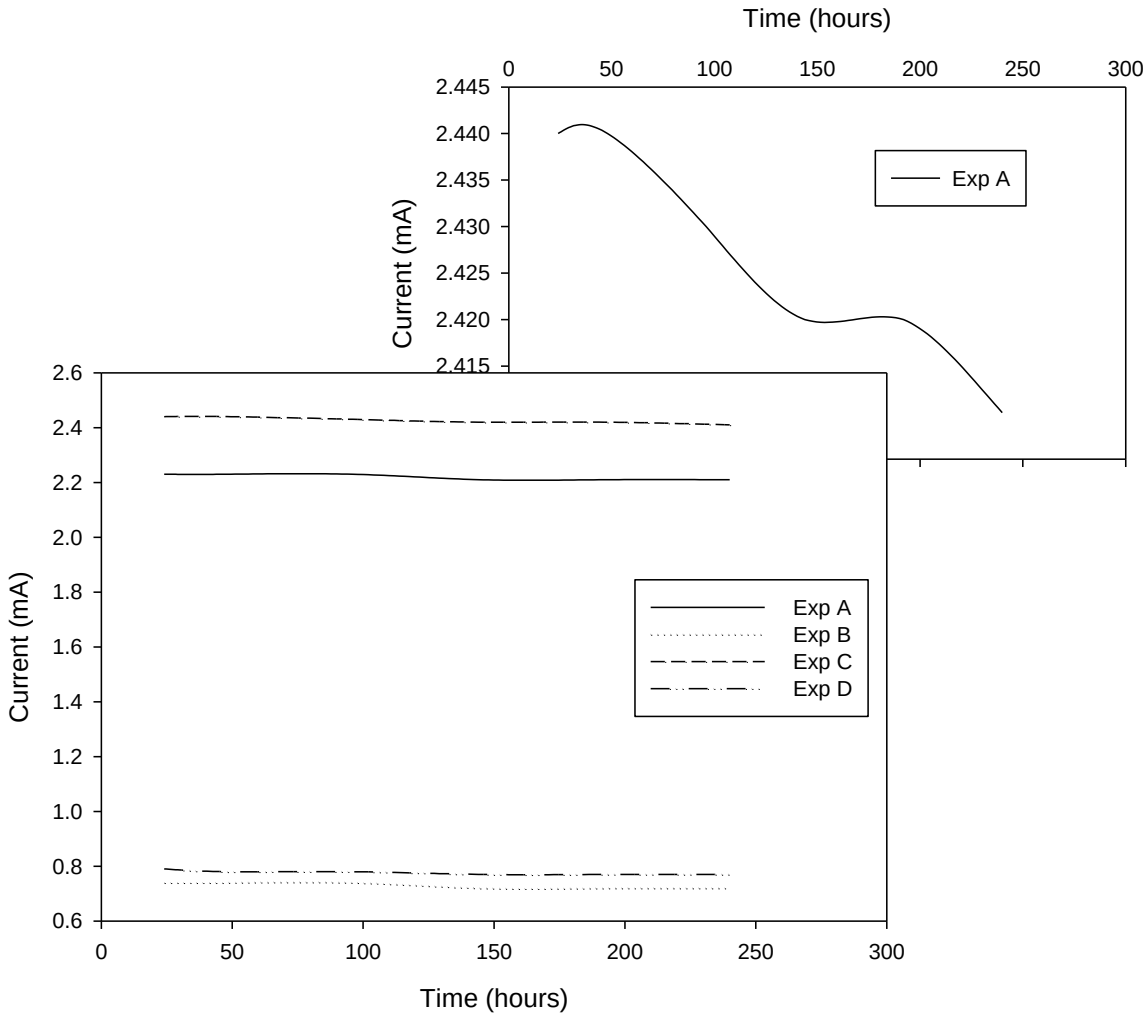


Figure 5. 2. Time course of current during the electrokinetic process

5.1.3 In situ microbial growth

Figure 5.3 and 5.4 show a substantial number of bacteria in different sections of the soil matrix. This is an indication that the bacteria were able to survive under the application of the electric field considering electroosmosis, pH, electrical potential and temperature variations can lead to the death of the microorganisms due to the electro-halo-thermal environment that may not favour microbial survival by damaging their cell membranes (Lear et al., 2007). Bacteria growth was not inhibited by the electric field since the bacteria showed normal growth variations with time. The viable cells in every particular section of the soil matrix were rather greatly influenced by the pH (Figure 5.5). The bacteria were able to move to the electrode wells and the viable cell counts increased with time as

opposed to the beginning of the experiment. Regarding movement, the bacteria in the system is mostly affected by electroosmosis because it's their main transport system much as they are also transported by electrophoresis (Kim et al., 2010, Mena et al., 2016). Figure 2 shows that there are substantial variations in the cell counts along the normalised distance from the cathode. It is known that bacteria can grow under a wide range of pH values but the optimum pH conditions for *Pseudomonas aeruginosa* are pH 7 (Das and Mukherjee, 2007). With highly variable pH gradients in the bio-electrokinetic reactors ranging from as high as 11.78 to as low as 2.3 the highest colony forming units were identified in sections of the soil matrix whose pH was between 9 and 6. These were areas between 50 mm and 100 mm normalised distances from the cathode. The strong growth patterns also indicate that the bacteria contributed to the degradation of the hydrocarbons by utilising the organic compounds as substrate leading to a 71.4 % highest reduction in total carbon in experiment A from 0.238 mg of carbon/mg of soil to 0.068 mg of carbon/mg of soil (Figure 5.6). The experiments that had the highest oil recovery had the lowest carbon content in the soil since there was a reduced number of hydrocarbons for the microbes to degrade. Figure 5.7 also goes on to show that after 240 hours the content of carbon in each of the sections of the soil influenced the colour of the soil after drying in all experiments. The samples with a darker colour had more carbon content as compared to those that are brighter. There was a reduction in carbon content starting from sample A towards sample E with E having the least carbon content. From there on the carbon content starts increasing from sample F to H with H having the highest carbon content after 240 hours (Figure 5.7). These observations were consistent in all the experiments carried out in this research.

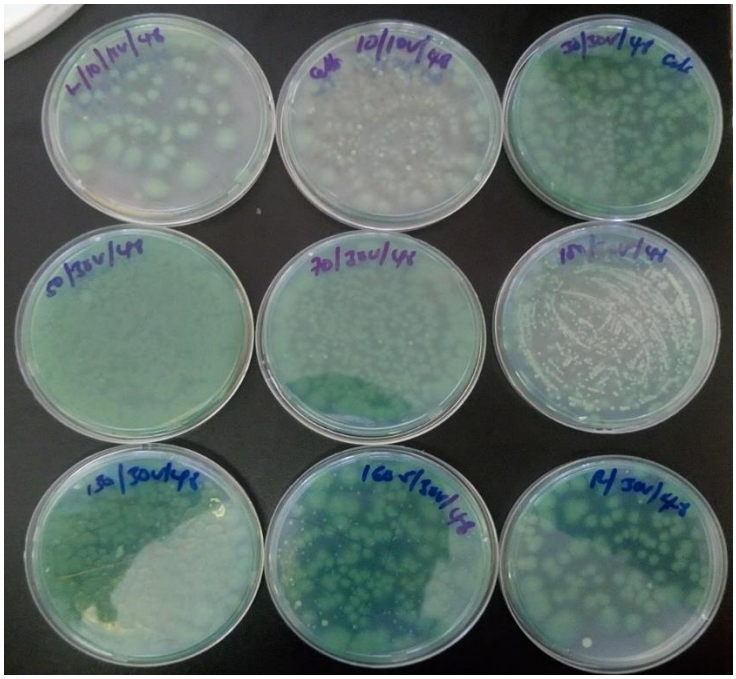


Figure 5. 3. Plate count agar plates showing the dominance of the *Pseudomonas aeruginosa* strain after 48 hours of running

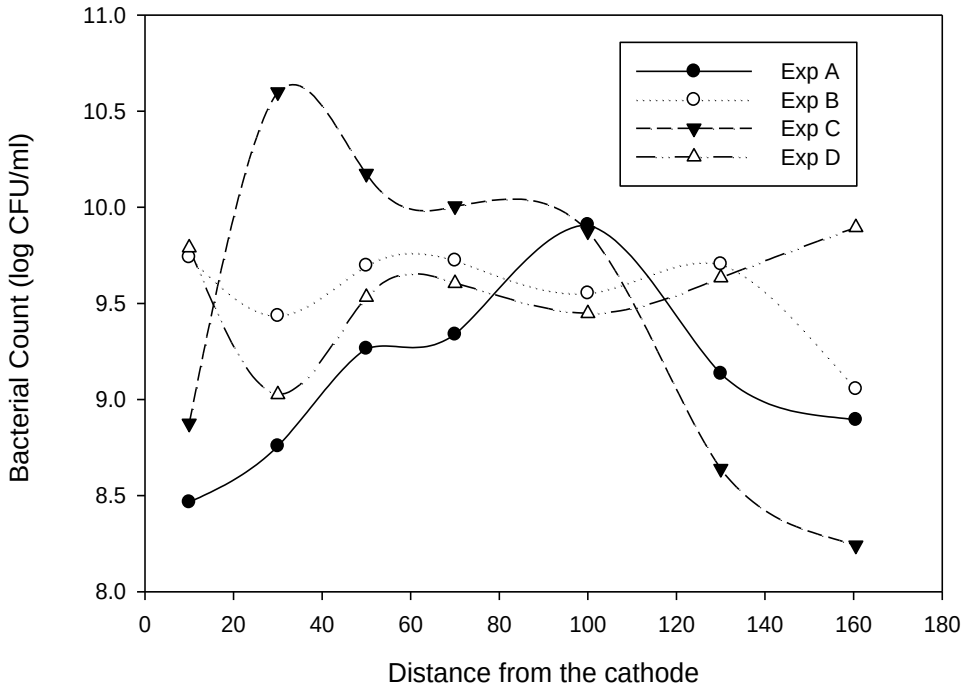


Figure 5. 4. Average bacterial counts to the end of the experiment.

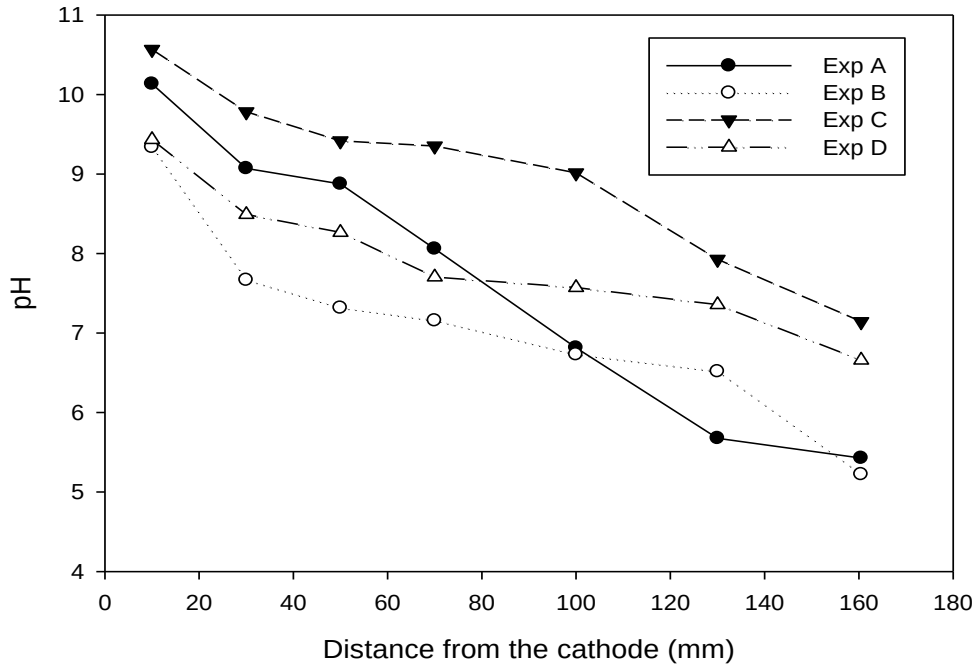


Figure 5. 5. Average pH distribution up to the end of bio-electrokinetic treatment.

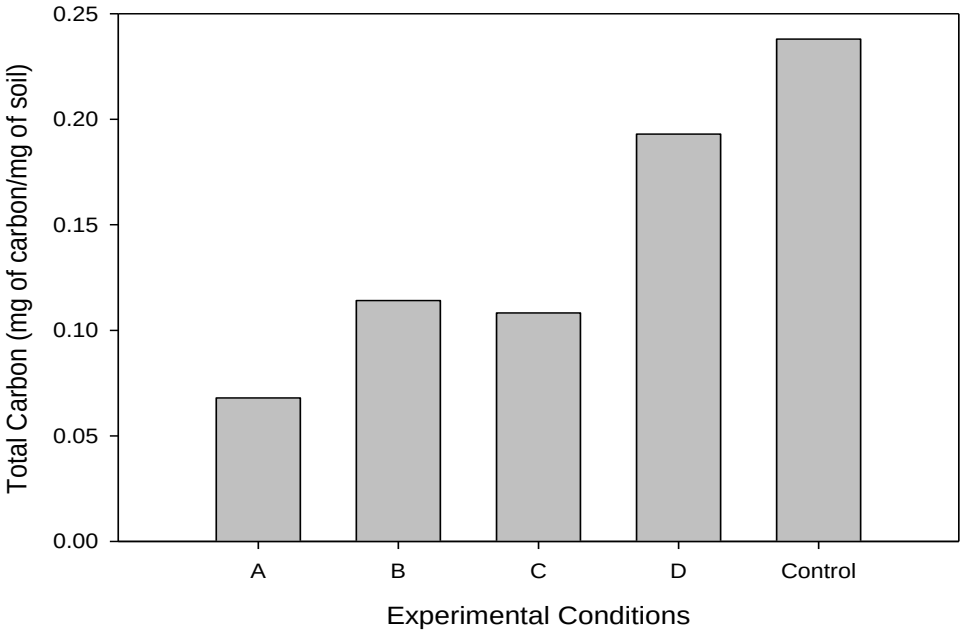


Figure 5. 6. Total Carbon remaining in the soil matrix after the experiments

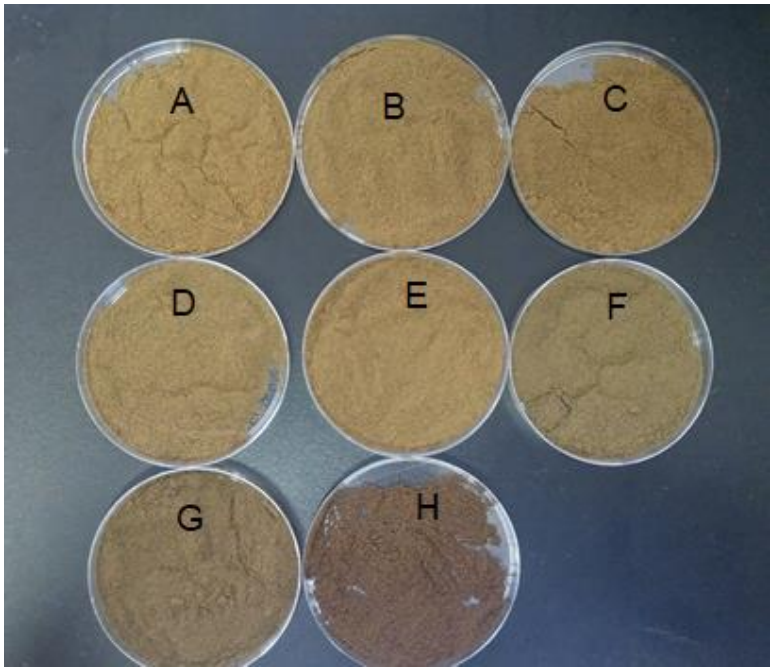


Figure 5. 7. Soil samples collected from the bioelectrokinetic reactor having different colours symbolizing the difference in carbon content left in the different sections of the medium after 240 hours; A-10 mm, B-30 mm, C-50 mm, D-70 mm, E-100 mm, F-130 mm, G-150

Conclusion

Biosurfactants have the capacity to accelerate oil recovery in the electrokinetic process but the system is mostly affected by the voltage gradient since the highest voltage had the highest oil volume recovered. EOF of oil is possible but is highly affected by the filter pores and oil viscosity. The survival and growth of bacteria under the electric field applied gives promising results for in situ biosurfactant production. A study is however being made to substantiate the effect of different biosurfactant concentrations to the process.

CHAPTER SIX

BIOELECTROKINETIC REMEDIATION WITH DIFFERENT SPECIFIED ELECTRODE CONFIGURATION STRATEGIES

6.1 Background

In the past decades electrokinetic remediation has emerged as a promising technology in effective decontamination of soil (Shen et al., 2007). Electrodes can however either inhibit or enhance the removal of pollutants by either decreasing or increasing electroosmotic flow/electric current flow respectively. For example, electrode spacing is reported to affect the oil phase and water phase contents in the liquid phase differently which may directly affect current flow and electroosmotic flow (Yang et al., 2005). Approaching electrodes much as narrowly studied have been used at times as an effective method to improve the efficiency of contaminant removal in the system by decreasing the pH, increasing electromigration hence improving the process of remediation. In on-site electrokinetic remediation, great emphasis is put on the configuration of the electrodes besides consideration of voltage gradient, electrolyte and operational time because electrode configuration strongly affects the final efficiency of the process and the overall costs (Kim et al., 2014b). Because so little has been studied about the impact of electrode configurations on the electrokinetic method of remediation, this research is meant to study the effects of different electrode configurations on oil recovery, electroosmotic flow, pH and the general efficacy of the process.

To study the effects of electrode configurations on the remediation process, graphite electrodes (100 mm long × 20 mm diameter) were located into the electrode compartments to have three distinct experiments resulting from fixed electrode spacings of 185 mm, 260 mm and 335 mm while the fourth involved continuous movement of electrodes to have spacings of 335 mm, 260 mm and

185 mm as a continuous approaching electrode configuration with all the electrodes connected to the DC power supply of 30 V. The three distinct experiments with fixed electrode spacings of 185 mm, 260 mm and 335 mm were carried out for 10 days each with 2 kg of contaminated soil inoculated with 30 g of cells and 200 mL of cell free biosurfactant supernatant with 22 g/L of biosurfactant. In the fourth set up electrode distances were reduced every after 80 hours from 335 mm, to 260 mm to 185 mm with 2 kg of contaminated soil inoculated with 30 g of cells and 200 mL of cell free biosurfactant supernatant with 22 g/L of biosurfactant. All the experiments were run in triplicates.

6.2 Variation in soil pH and microbial counts

To make a proper assessment of the transportation of particular species in an electrokinetic system, it is necessary to consider their behaviour in an environment with widely varying pH values (Ouhadi et al., 2010). To study the variations in pH and its effect on the species in the system, pH and microbial counts were determined in seven different sections of the reactor away from the cathode at 10 mm, 30 mm, 50 mm, 70 mm, 100 mm, 130 mm and 160 mm every after 48 h for 240 h. Figure 6.1 shows the variations in pH and microbial counts as averages for 240 h. For analytical purposes 335 mm and 185 mm spacing were used in this case. The electrode spacing of 335 mm produced a pH range between 10.14 to 1.95 while that of 185 mm was between 11.06 and 5.26. The seemingly low pH values for 335 mm spacing contrary to the 185 mm spacing in the soil medium should have been as a result of the delayed encounter of the acid front and the alkaline front. When the electrodes are far apart like in the 335 mm electrode spacing, the two pH fronts have to move a longer distance before they can intersect, and this could even be more than the 240 hours for which the experiments were run. This means that the reactor area next to the cathode will be highly alkaline while the area next to the anode will highly be acidic until the two fronts intersect leading to a neutralisation phase that reduces the pH in the

cathode area (that currently has very high pH) and increases the pH in the anode area (that currently has very low pH). When the electrodes are closer to each other like in the 185 mm electrode spacing, the two fronts have a shorter distance to move leading to an early neutralisation phase that brings the pH in the soil nearer to pH 7 than in the 335 mm electrode spacing. Indeed Figure 6.1 shows the that the average pH of the soil in the 185 mm electrode spacing is nearer to 7 as compared to the 335 mm electrode spacing which has very high pH in the area next to the cathode and very low pH in the area next to the anode meaning the two pH fronts had not intersected to cause a neutralisation phase. This is because in an electrokinetic reactor oxidation reactions occur at the anode and reduction reactions occur at the cathode. These reactions lead to the formation of the acid front at the anode and an alkaline front at the cathode on the immediate application on an electric field. But as ions start migrating, the pH dynamically changes across the system as the H^+ move towards the cathode. With H^+ almost twice mobile (1.75 times) as OH^- from the cathode, the protons dominate resulting into movement of the acid front towards the cathode where H^+ meet OH^- and form water (Shu et al., 2015). The pH in the system is, therefore, dependant on the movement of H^+ and OH^- across the system (Cameselle et al., 2013).

On the other hand, it has been reported that besides electroosmosis, electrical potential and temperature variations, pH can lead to the death of the microorganisms due to the electro-halo-thermal environment that may not favour their survival (Lear et al., 2007). From Figure 6.1 it is evident and understandable that the highest microbial counts were noticed under the electrode spacing of 185 mm which produced the most favourable conditions for bacterial growth considering the optimum pH conditions for *Pseudomonas aeruginosa* is pH 7 and indeed the highest counts were recorded at a pH nearest to 7. The high acidity of the soil produced under 335 mm due to higher oxidation reactions at the anode inhibited cell growth in the medium.

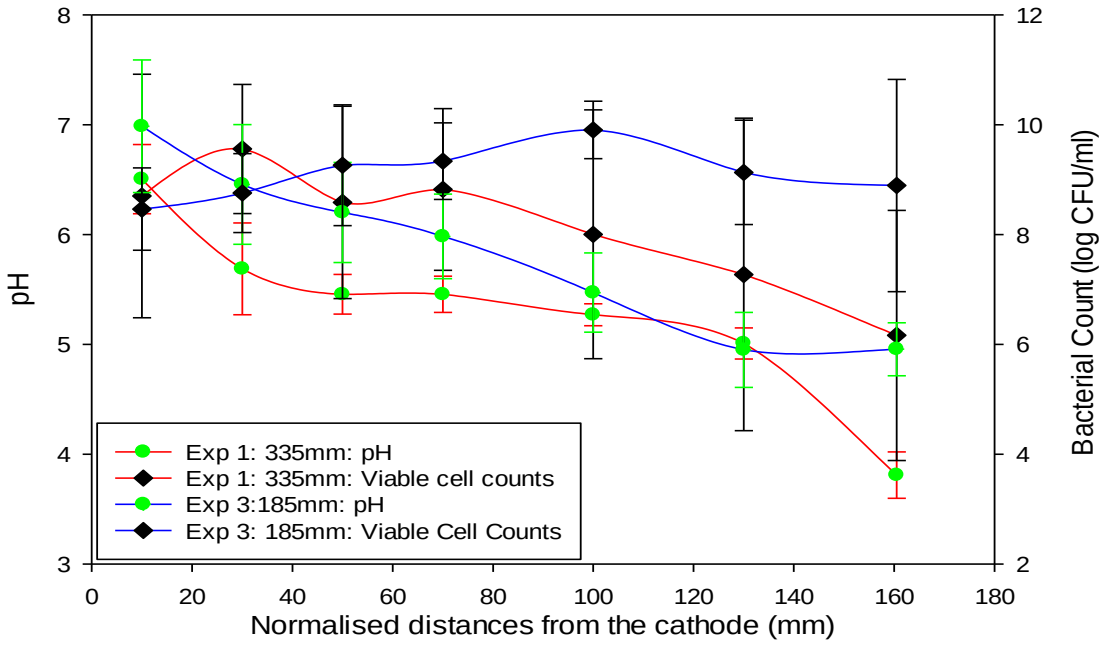


Figure 6. 1. PH and bacterial count variations in different sections along the normalized distance from the cathode

6.3 Current and oil recovery

Much as there was no significant difference in the current conducted through the electrolyte, the 185 mm spacing conducted the highest current through the electrolyte as shown in Figure 6.2. In experiments with 260 mm and 335 mm electrode spacing, current increased to 2.32 and 2.29 mA respectively in the first 96 hours and gradual reduction was there after observed while in the 185 mm experiments, the highest current (2.39 mA) was observed after 144 h before a gradual reduction was noticed. In most electrokinetic reactors electric current increases quickly during the first few hours and then gradually thereafter. This is usually due to resistance in the interface between electrodes and the electrolyte which increases because of concentration polarization and water dissociation. The other reason is usually because ions with positive or negative charges move to the two ends of the electric cell as a consequence of electrodialysis, which results in the drop of ionic strength in soils and the current (Wang et al., 2007).

This explains why the 335 mm spacing reaches the highest current peak first considering the acidic conditions accelerate the disassociation of water in the oxidation reactions at the anode resulting into a quicker increase in the ionic strength of the reactor contrary to the 185 mm spacing whose anode pH is higher.

A combination of biosurfactants and the application of an electric field in this study was meant to accelerate the rate of oil recovery in the system by having a combined effect of electro-demulsification and biosurfactant activated demulsification. Biosurfactants were applied to act as flushing agents because of the low solubility and hydrophobicity properties of organic contaminants, which makes them complex to remove from the solid matrix while the electric field promotes electro-demulsification and electro-coalescence of small droplets so as to have an efficient phase separation (Elektorowicz et al., 2006). The highest oil recovery was observed during the 185 mm (fixed) and the continuous 335, 185, 260 mm electrode spacing as opposed to the 260 mm and 335 mm fixed electrode spacing. The average total oil recovered was 74.65 %, 54.625 %, 53.7 %, and 61.65 % for fixed electrode spacing of 185 mm, 260 mm, 335 mm, and the continuous electrode spacing of 335, 260, 185 mm respectively. The highest oil electroosmotic flow was towards the anode compartments with the highest of all still happening under the 185 mm spacing (Figure 6.3). This is in agreement with the reports made by Yang et al. (2005) who observed the highest total oil recovery and oil electroosmotic flow with the smallest electrode spacing of 4 cm. These findings indicate that spacing's that lead to the high and quick electromigration of ionic species in the system lead to the increase in ionic strength which also enhances the conduction of current in the system. The conduction of high current through the system gives the highest oil recovery and oil electroosmotic flow since electroosmotic flow, electro-demulsification and electro-coalescence are directly proportional to the electric field.

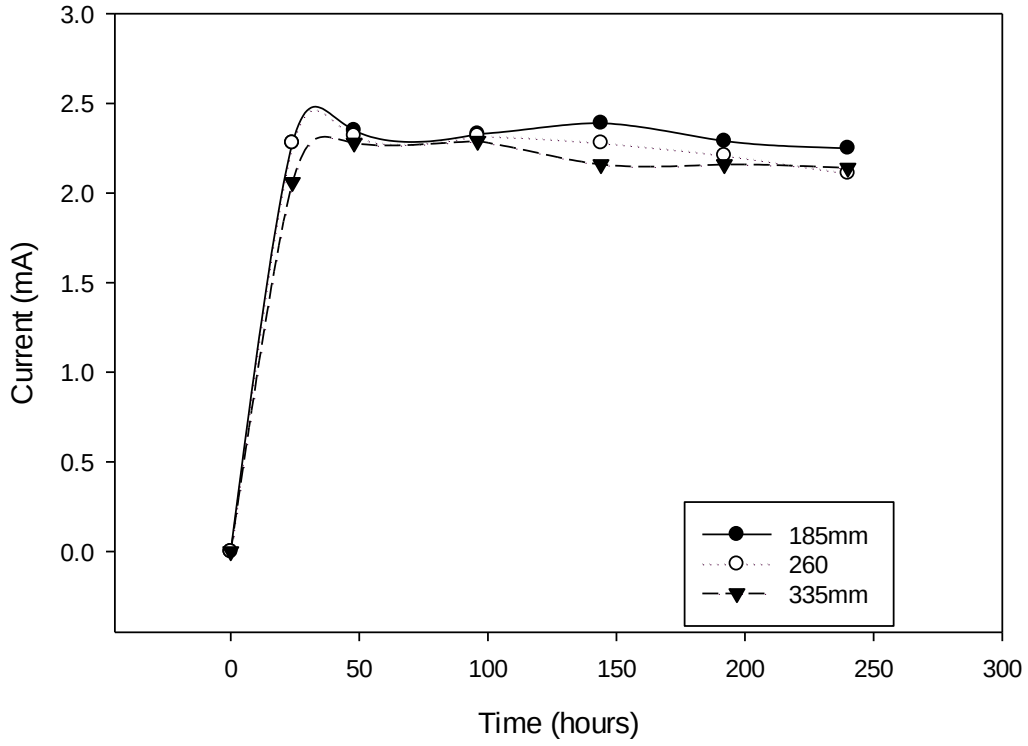


Figure 6. 2. Time course of current for 185, 260- and 335-mm electrode

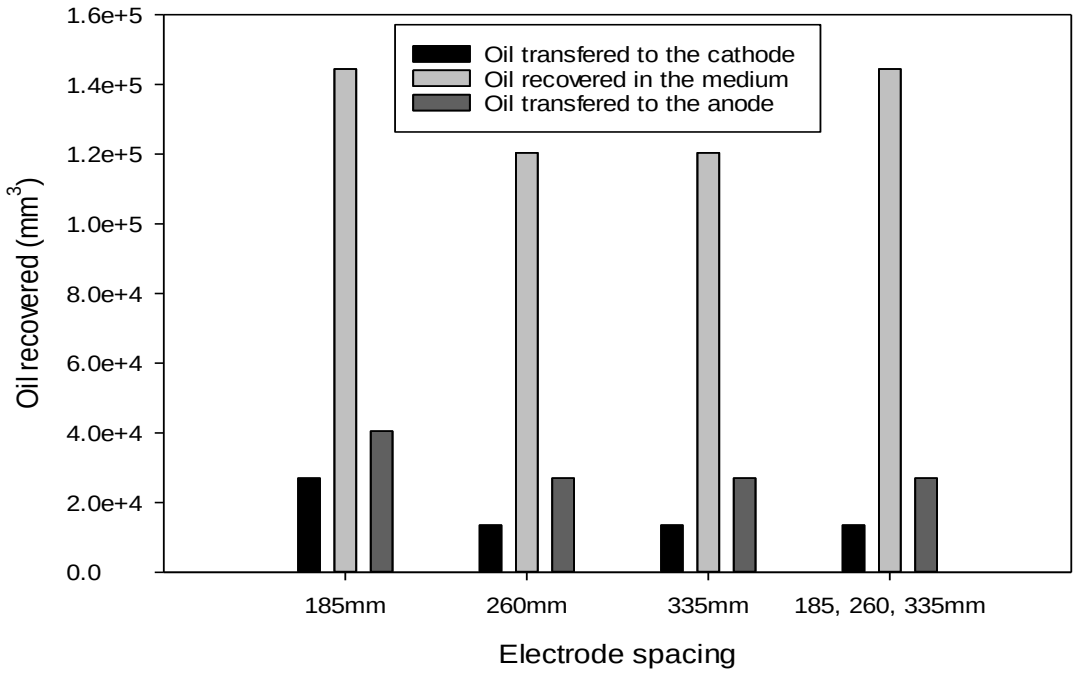


Figure 6. 3. Oil recovery for different electrode spacing

6.4 Electroosmotic Flow (EOF)

Electroosmosis affects oil recovery but is also affected by various factors including current, viscosity, ionic concentration, temperature, dielectric constant of the interstitial fluid and surface charge of the solid matrix (Cameselle et al., 2013). Current for example is directly proportional to EOF considering Helmholz–Smoluchowski theory. The differences in current were insignificant especially from the beginning of the experiment to 100 hours. Between 100 hours and 200 hours, there was a more observable difference which could have led to the minor differences in the differences in oil recovered. Figure 6.4 shows the continuous 335, 260, 185 mm approaching electrodes producing the highest EOF followed by the 185 mm fixed electrode spacing. These two experiments produced the highest average current of 2.41 mA and 2.39 mA respectively. In Figure 6.4 water EOF as a function of time against current for the continuous 335, 260, 185 mm electrode spacing is shown. The profile of EOF seems to meticulously follow that of current verifying the great dependence of EOF on the electric field in the first 24 hours but significantly drops until the anolyte is refilled in the anode compartment after 150 hours. It should also be observed from Figure 6.5 that the continuous approaching electrodes increase and maintain current flow in the system. On every reduction of the electrode spacing, the current increased with increase in electroosmotic flow. The same was also reported by Li et al. (2012) much as a different approaching electrode strategy from the one used here had been employed. The increase in current on every event of approaching electrodes or reduction in electrode distance is due to reduction in the electrolytic distance between the working electrodes. The new short electrolytic distance increases H^+ ions and high redox potential concentrations to quickly migrate to the cathode shortening the distance to be travelled by the low strength ions in the pore fluid that may have led to reduction in the current as a

result of resistance of the matrix or reactions between the migrating ions and the matrix species.

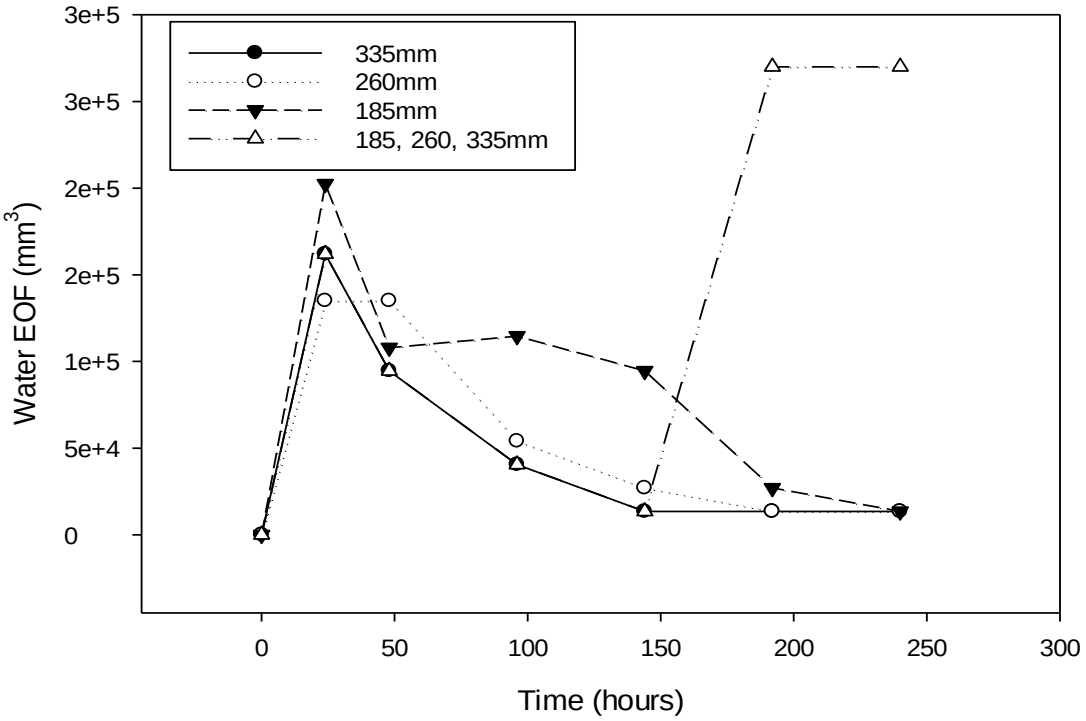


Figure 6. 4. Time course of EOF of water

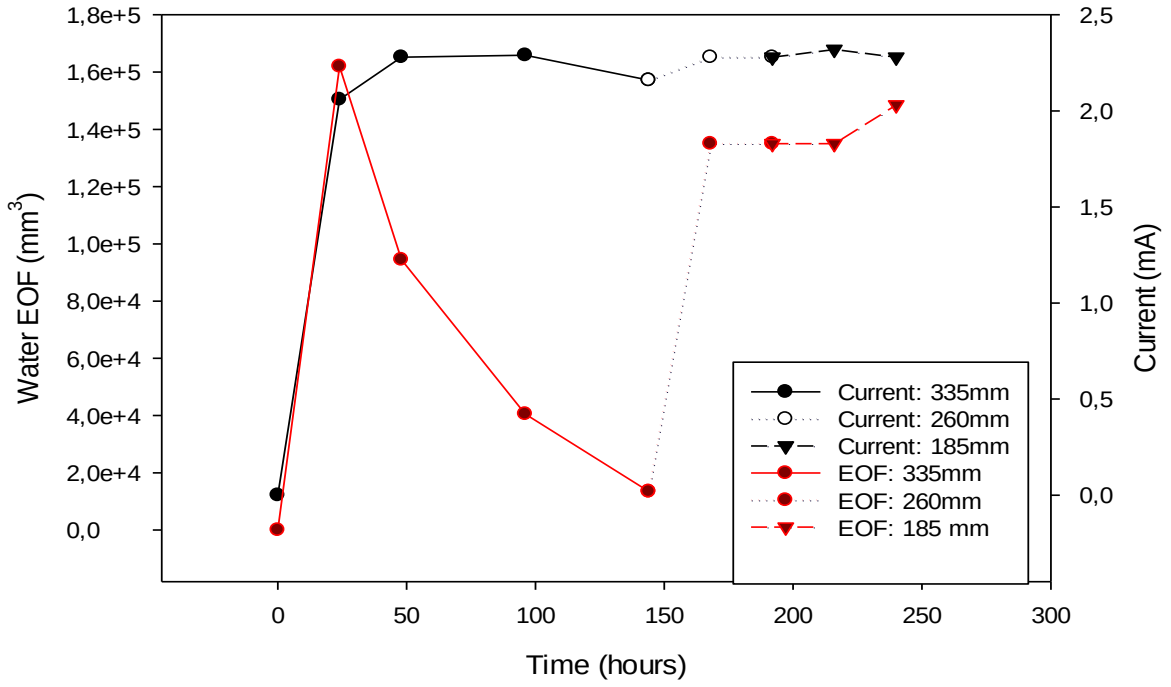


Figure 6. 5. Time course of EOF of water against the time course of current

6.5 Hydrocarbon removal after electrokinetic remediation

In the process of performing electrokinetic remediation, very few studies have been done about the effect of the electrokinetic process on the survival, growth, movement and enzyme activity of the microorganisms in the system (Lear et al., 2007). It has been well elucidated in section 6.2 above regarding the effect of electrode distance to the pH variations in the system which in turn affected the microbial counts in the system during operation for the 240 h. The initial carbon content in the soil as a result of spiking with petroleum hydrocarbons was 0.538 carbon/mg of soil before treatment. The analysis done using the Total Organic Carbon Analyzer showed that the 185 mm spacing had the lowest total carbon content with an average of 0.108 mg of carbon/mg of soil, followed by 260 mm with 0.1141 mg/mg followed by 335 mm with 0.133 mg/mg (Figure 6.6). It is obvious that the set up that facilitated the highest bacterial growth had the highest degradation. The values of total carbon remaining after 240 h were generally low

which could indicate the duty played by the biosurfactants in increasing the pollutant bioavailability to the degrading microbes.

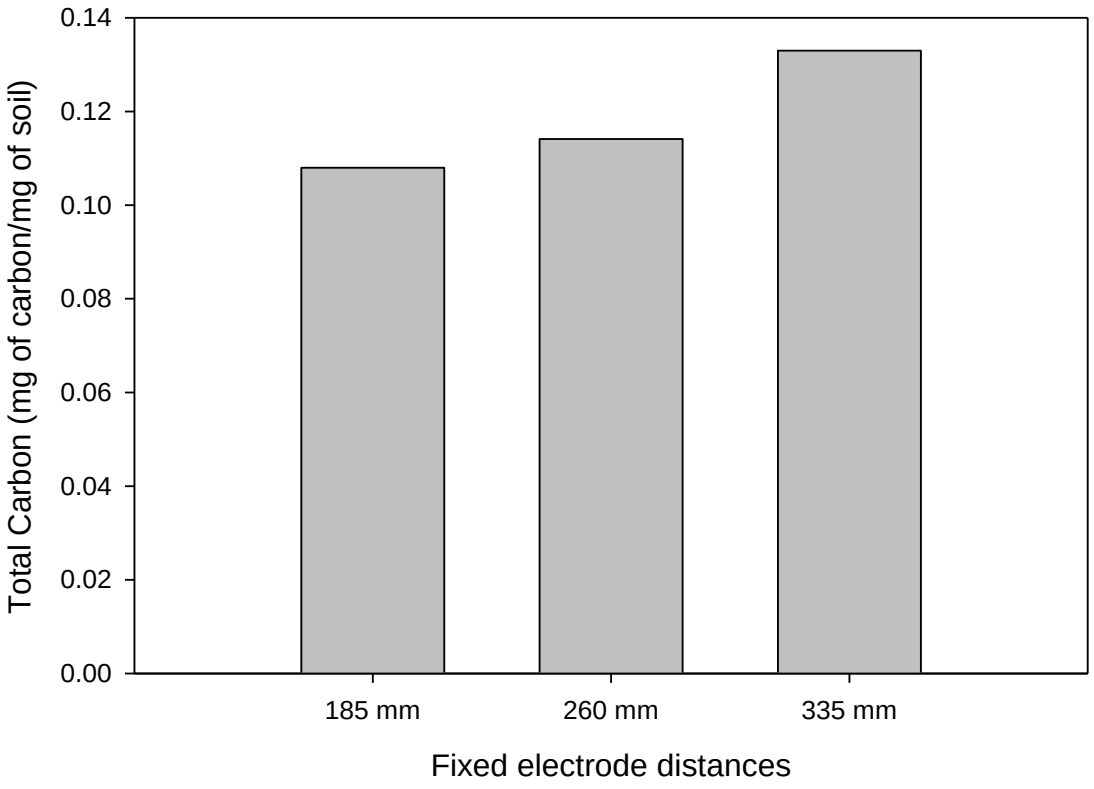


Figure 6. 6. The average total carbon remaining in the soil after 240 hours of the experiment

Conclusion

Approaching electrodes or conventional reduction in electrode spacing has the capacity to increase and maintain high current flow, electroosmotic flow and stabilize pH which may favor oil recovery, bioremediation and electrokinetic remediation as compared to fixed electrodes. The lowest electrode spacing is however most likely to increase the electrokinetic remediation efficiency as compared to larger electrode spacings.

CHAPTER SEVEN

BIOELECTROKINETIC REMEDIATION OF OIL CONTAMINATED SOIL WITH INCREASING BIOSURFACTANT CONCENTRATIONS

7.1 Background

Organic contaminants have low hydrophobicity and solubility properties, it is usually complex to remove them from a solid matrix unless a surfactant is applied to act as a flushing agent (Boulakradeche et al., 2015). The most dangerous organic pollutants are usually insoluble in water, non-ionic and have non ionisable molecules which makes it impossible to remove with the two main processes of electromigration and electroosmosis (Cameselle et al., 2013). The removal of organic contaminants in this case requires simultaneous adequate electroosmotic flow and solubilisation of the contaminants which can only be achieved by use of solvents and surfactants otherwise electrokinetics has to applied in combination with another method such as chemical oxidation and permeable reactive barriers (Cameselle et al., 2013). In our studies we applied biosurfactants to increase the solubility and mobility of the contaminants in the electrokinetic system (Virkutytea et al., 2002, Reddy and Saichek, 2004). The solubilisation capacity of the biosurfactant is therefore very important for this to be achieved (Giannis et al., 2007). Through micellisation, surface tension reduction and solubilisation and increased adsorption, biosurfactants would be expected to increase the rate of contaminant removal by altering the surface properties of the matrix leading to an enhanced demulsification and electroosmotic flow (Gomes et al., 2012, Reddy and Shirani, 1996, Alcantara et al., 2010).

To conduct studies for the evaluation of biosurfactant concentration on the remediation process, 2 kg of soil spiked with oil were mixed using an overhead stirrer with 30 g of bacterial cells produced for 24 hours and with either 26 g/L,

56 g/L or 84 g/L of biosurfactants to have three distinct experiments based on different biosurfactants concentrations.

7.2 Current

Figure 7.1 below shows the relationship between current and time from the beginning to the end of the experiments. The experiment with 28 g/L of biosurfactants had the highest current of 2.4 mA conducted through the electrolyte followed by 84 g/L with 2.39 mA and lastly 56 g/L with 2.3 mA. Much the theoretical expectation would be that the experiment with the highest biosurfactant concentration would have the highest current conducted because of the polar chain of the biosurfactants, this was not really the case. The insignificant differences in the current experienced in the three experiments can be attributed to the differences in the amounts of the natural ionic species in the soil that was treated. The natural ionic species in the soil therefore had more influence on the ionic strength of the experiments overcoming the differences that should have been brought about by the differences in biosurfactant concentration. The current in all the experiments started low at the beginning of the experiment then gradually increased and later started decreasing towards the last days of the experiments apart from the experiment with 28 g/L of biosurfactants. The increase in the current at the beginning of the experiment is a result of disassociation of water producing OH^- ions at the cathode and H^+ ions at the anode which besides the other ion species in the soil increase the ionic strength of the system leading to increase in current (Cameselle et al., 2013). The decrease in the current towards the end of the experiment is due to resistance in the interface between electrodes and the electrolyte which increases because of concentration polarization and water dissociation (Wang et al., 2007). The other reason is because ions with positive or negative charges move to the two ends of the electric cell because of electrodialysis, which results in the drop of ionic strength in soils and the current. (Wang et al., 2007). On the other hand, the increase in current for

28 g/L after 144 hours was because of the increase in ionic strength which was due to the addition of the electrolyte in the anode compartment which was running out before the end of the experiment.

7.2 Electroosmotic Flow (EOF) and Electrophoresis

When an electric field is applied it leads to movement of the liquid phase (water and oil in this study) due to the existence of the electrical double layer at the interface of water/oil and the solid surface but also due to the migration of charged particles or ions in a colloidal system towards the counter charged electrode (Cameselle et al., 2013). Figure 7.1 shows the time course of EOF for 240 hours. Like current, EOF starts low and then increases gradually in the first days of the experiment. The highest EOF after 48 hours was observed in the experiment with 28 g/L of biosurfactants with 378,000 mm^3 of water moving from the anode to the cathode followed by 56 g/L with 229,500 mm^3 and lastly 135,000 mm^3 in the 84 g/L. EOF is directly proportional to current according to Helmholtz–Smoluchowski theory The results represented in Figure 7.1 illustrate the relationship between current and EOF in agreement with Helmholtz–Smoluchowski's theory where current is directly proportional to EOF. The increase in current was followed up by a proportional increase in EOF with the vice versa being true. The experiments with the highest biosurfactant concentration had the lowest EOF. This is because the EOF was inhibited by the movement of biosurfactants which moved from the medium compartment towards the anode compartment. The movement of the biosurfactants towards the anode increases the resistance to EOF at the medium-anode interface as the water moves in the opposite direction from the anode compartment towards the medium compartment. This explains why EOF from the anode compartment towards the medium compartment (flow of water) decreased with an increase in biosurfactant concentration. After 48 hours, the anolyte remaining in the anode compartment started becoming turbid due to the movement of negatively charged colloidal

particles from the soil medium to the anode compartment. These particles coagulated in the anode compartment forming flocs (Figure 7.2). The flocculation of these colloids led to their sedimentation at the bottom of the reactor. The movement of the colloidal particles was mainly seen towards the anode because of the surface nature (negatively charged) of the soil from which the colloids were migrating from (Yang et al., 2005).

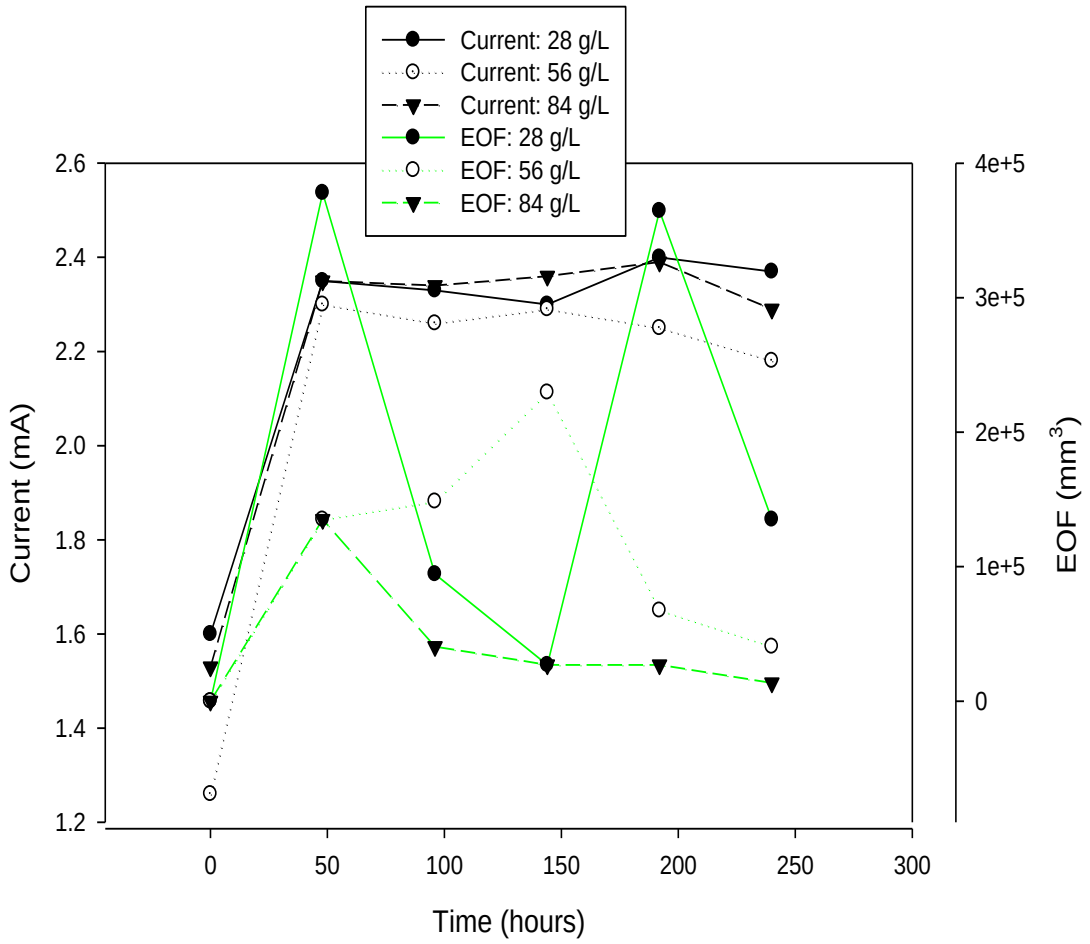


Figure 7. 1. Time Course of current and EOF flow in the reactor for 28 g/L, 56 g/L and 84 g/L biosurfactant amendments.

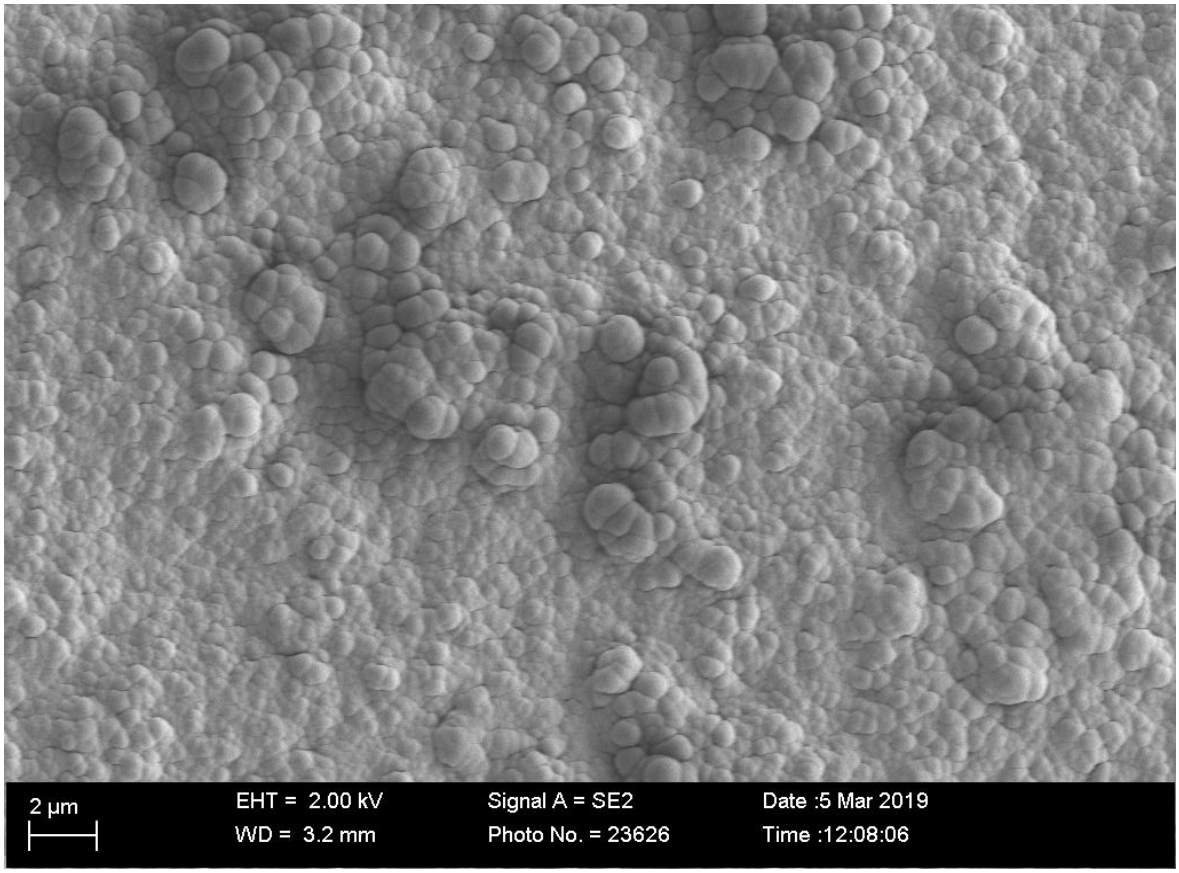


Figure 7. 2. SEM micrograph of coagulated colloidal particles in the anode compartment after electrophoresis

7.3 Zeta potential of the biosurfactants under different pH

Zeta potential is important in understanding the state of the surface and stability of surface-active substances such as biosurfactants. The biosurfactants produced were subjected to zeta potential analysis to determine their surface charge, stability and surface modifications under the alkaline pH of 13, neutral pH of 7 and acidic pH of 1 which were also the pH ranges experienced in the bioelectrokinetic reactor. The zeta potential results (Table 7.1) show that at pH 1, the zeta potential is positive at 0.085 mV, -14.5 at pH 7 and -17.4 at pH 13. With increased adsorption of H^+ ions on the surface of biosurfactants in acidic conditions, the zeta potential increased at pH 1 becoming more positive while at pH 7 and pH 13, the adsorption of OH^- ions on the surfaces of the biosurfactants made the surface charge become more positive (Narong and James, 2006). Since

the values obtained are between -25 mV and 25 mV this represents the unstable nature of the biosurfactants between pH 13 and 1. This instability results in aggregation of the biosurfactants in their final destination at the anode compartment due to van der waals interactions (Narong and James, 2006). In the reactor, the lowest pH is experienced at the anode while the highest pH is experienced at the cathode. These results are also an indication that the biosurfactants moved from the medium compartment which was between pH 10 and 4 to the anode compartment due to the strong negative surface charge of the biosurfactants. When the biosurfactants reach the anode compartment, they aggregate because of their unstable nature to form a continuous phase (Figure 7.3). The negative polarity of the biosurfactants is also a confirmation that the surface-active agents are rhamnolipids (Rikalovic et al., 2012).

Table 7. 1: Zeta potential of the biosurfactants in pH at pH 13, 7 and 1

pH	**Zeta Potential mV**			
	Sample 1	Sample 2	sample 3	Average
pH 1	-1.86	2.16	-2.15	0.08533333
	1.93	-1.87	-0.564	
	0.749	2.14	0.233	
pH 7	-14.6	-14.4	-14.1	-14.522222
	-12.9	-13.6	-15.7	
	-14.7	-15.4	-15.3	
pH 13	-15	-17.5	-17.2	-17.422222
	-18.6	-18.7	-16.1	
	-19.5	-14.9	-19.3	

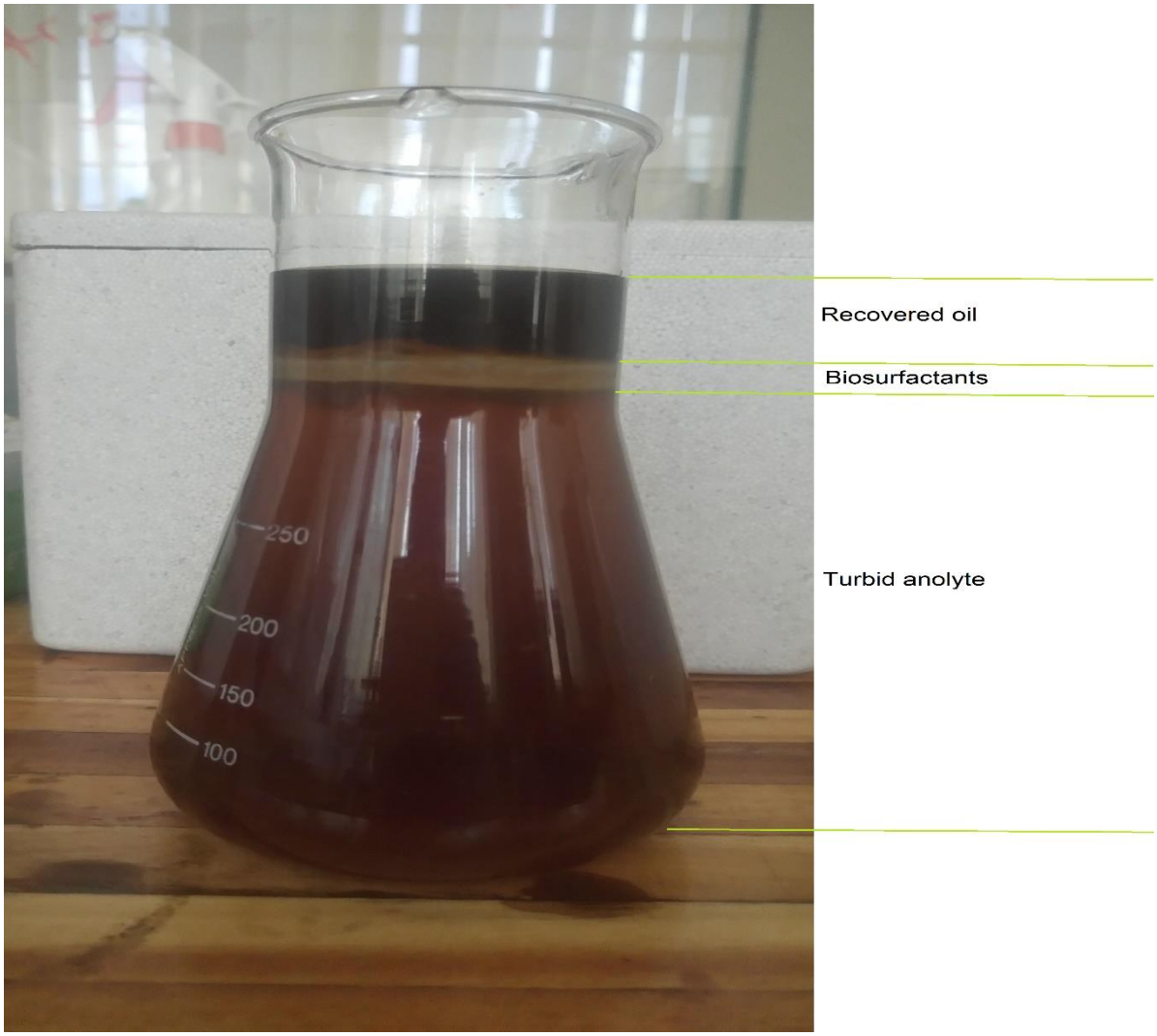


Figure 7. 3. Recovered oil, biosurfactants and a turbid electrolyte collected from the anode compartment after the experiment

7.4 Oil recovery

In this study biosurfactants were applied to enhance the process of oil recovery from the soil by combining the efforts of electro-coalescence, electroosmosis and the flushing properties of biosurfactants to attain improved efficiency of the process. In Figure 7.4, the highest oil recovery was observed in the experiment with 56 g/L of biosurfactants with 249,475 mm^3 of oil recovered followed by 84 g/L with 163,700 mm^3 while 28 g/L only recovered 145,462.5 mm^3 of oil. These results show that the increase in biosurfactant concentration from 28 g/L to 56 g/L led to a proportional increase in oil recovery but the further increase to 84 g/L

did not cause a further increase in oil recovery. The hyper increase in biosurfactant concentration for the purpose of increasing demulsification has been reported to have insignificant effect with further increase producing minor improvements in demulsification efficiency (Rocha et al., 2017, Hajivand and Vaziri, 2015). The lower oil recovery observed with 84 g/L of biosurfactants compared to the 56 g/L should be attributed to the extreme growth stimulation of the microbes due to the increase in bioavailability. Indeed with 84 g/L of biosurfactants, Figure 7.6 below shows the extreme growth of the microbes in the first 100 hours followed by a reduction in the microbial counts thereafter. The extreme growth of the bacteria in the first 100 hours led to the utilisation of some of the available biosurfactants due to high competition for a carbon source by the microbes. With the biosurfactants being utilised by the microbes, the demulsification activity of the biosurfactants reduced due to the reduction in biosurfactant concentration in the system leading to lower oil recovery. Most of the oil recovered stagnated in the medium compartment with miniature amounts moving to the electrode compartments with the highest percentage moving towards the anode compartment. The higher the amount of oil recovered in the medium compartment was, the higher the amount of oil that moved to the electrode compartments. Oil that moved to the anode compartments in the experiment with 56 g/L of biosurfactants was 94500 mm^3 followed by 84 g/L with 54000 mm^3 and lastly 28 g/L with 27000 mm^3 (Figure 7.4). Much as it is expected that the liquid phase (oil and water) was supposed to move to the cathode as a result of electroosmotic flow (Yang et al., 2005), this was not the case because electroosmosis was affected by viscosity and the molecular size of the water and oil. The larger the size of the molecules the lower the electroosmotic rate since the liquid phases may not easily go through the filter to the electrode chambers (Yang et al., 2005). This competition between molecules is most experienced at the cathode-medium compartment interface as compared to the anode-medium compartment due to the direction of electroosmotic flow which is

towards the cathode. The competition at the anode-medium compartment further decreases after the reduction of electroosmotic flow from the anode to the medium compartment. After reduction of EOF at the anode-medium interface, the oil molecules tend to move towards the anode since the oil molecules are left to compete with only colloidal particles which at the time were also moving through the filter to the anode due to electrophoresis. The oil molecules are then able to out compete the colloidal particles hence end up in the anode compartment as compare to the cathode compartment resulting in higher volumes of oil collected at the anode.

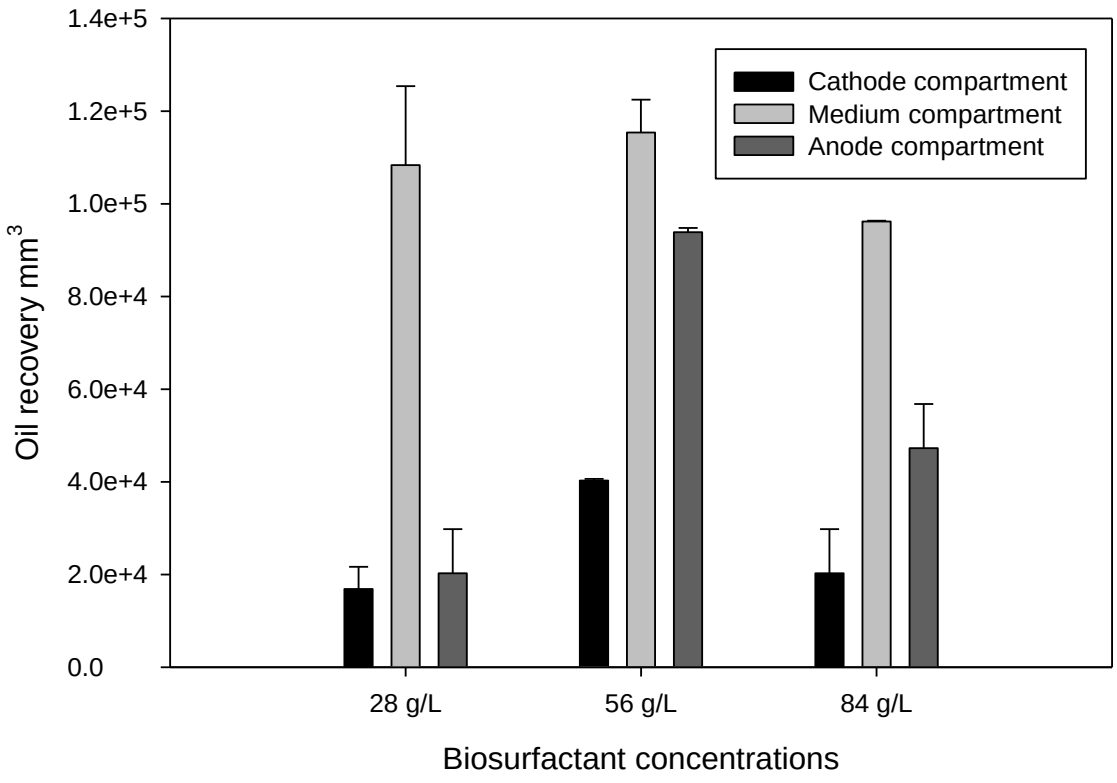


Figure 7. 4. Oil recovery and movement for different biosurfactant concentrations of 28 g/L, 56 g/L and 84 g/L after 240 hours.

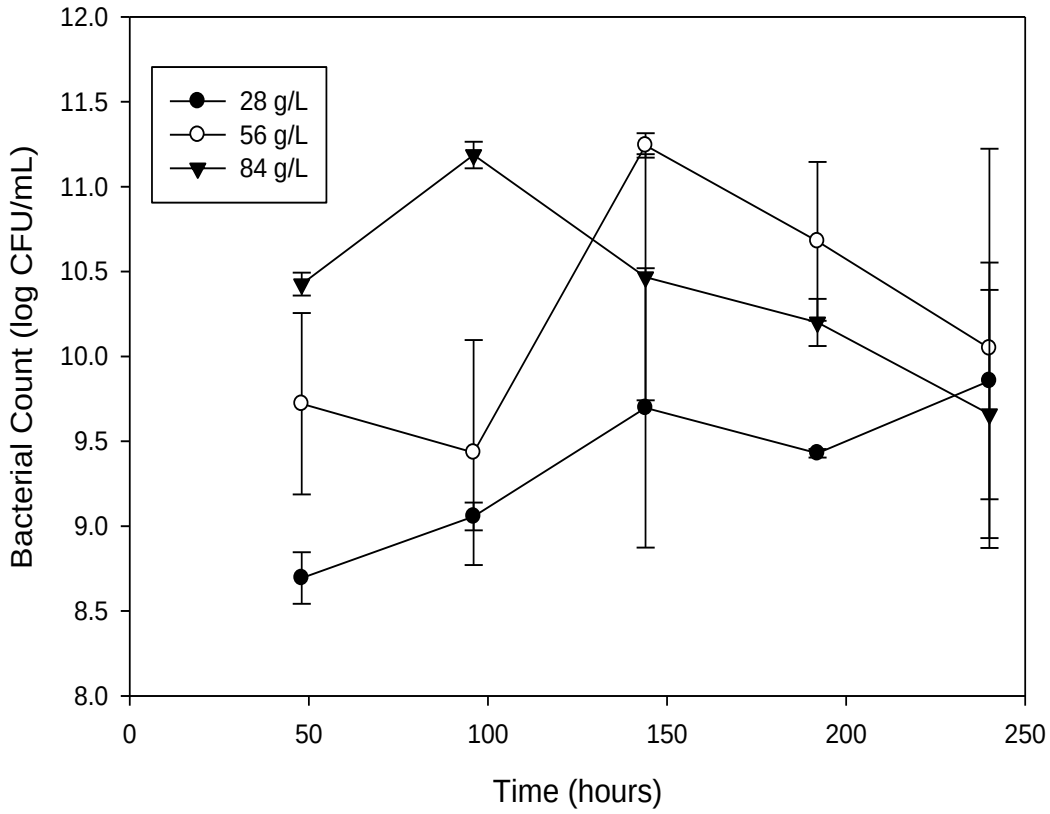


Figure 7. 5. Time course of bacterial growth for 240 hours.

7.5 PH

The highest pH registered in the experiment with 56 g/L, 26 g/L and 84 g/L of biosurfactants was 11.54, 11.45 and 11.92 respectively (Figure 7.6). The lowest average pH values observed were also 5.79, 5.4, 6.19 respectively. The highest pH values were observed in the cathode compartments and the lowest in the anode compartments. The range of the pH in all the experiments is closely related. This means that biosurfactants did not have substantial effect on pH that could result from different interactions between the biosurfactants and other chemical species in the reactor. The high pH values observed at the cathode are due to OH^- ions produced from reduction reactions in the cathode compartment while the low pH values at the anode are due to production of H^+ ions in the anode compartment all resulting from disassociation of water (Cameselle et al., 2013, Shu et al., 2015).

These reactions lead to formation of the acid front at the anode and an alkaline front at the cathode on immediate application of an electric field. But as ions start migrating, the pH dynamically changes across the system as the H^+ ions move towards the cathode and OH^- ions towards the anode (Shu et al., 2015). The movement of theses ions produced a dynamic variation of pH across the system from high pH in areas near the cathode to low pH in areas the anode. With H^+ ions almost twice mobile (1.75 times) as OH^- ions from the cathode, the protons dominate resulting into movement of the acid front towards the cathode where H^+ meet OH^- (Cameselle et al., 2013). The movement of these ions produced a dynamic variation of pH across the system from very high pH in areas near the cathode to very low pH in areas near the anode.

7.6 Microbial counts

It has been reported that besides electroosmosis, electrical potential and temperature variations, pH can lead to the death of the microorganisms due to the electro-halo-thermal environment that may not favour their survival (Lear et al., 2007). To study the variations in pH and its effect on the species in the system, pH and microbial counts were determined in five different sections of the reactor away from the cathode. The variations in pH and microbial counts as averages for 240 hours are shown (Figure 7.6). 10.52 CFU/mL were the highest microbial counts determined in 84 g/L of biosurfactants, 10.14 CFU/mL were the highest in 56 g/L while 9.354 CFU/mL was the highest in 26 g/L. These results show that the increase in the concentration of biosurfactants led to increased bacterial growth stimulation in the system. Figure 7.6 displays the bacteria growth manifestations over time in which the bacterial growth patterns were characterised by vacillating and uneven growth patterns in the medium in all the three experiments from the beginning of the experiment to the end. The non-exponential growth patterns are due to the irresolute pH across the medium that keeps changing because of the migration of OH^- and H^+ ions as they try to reach

the electrodes of the opposite charge. Figure 7.6 shows the effect of pH on microbial growth. The most exclusive observation is that the location of the bacteria between 50 mm and 120 mm normalised distance from the cathode for the 28 g/L of biosurfactants led to the highest growth of bacteria as compared to the other sections of the reactor. Similarly, the highest growth patterns in 56 g/L and 84 g/L were both observed between 40 mm and 120 mm. It has been commonly reported that bacteria can grow under a wide range of pH values but the optimum pH conditions for *Pseudomonas aeruginosa* are pH 7 (Das and Mukherjee, 2007). With highly variable pH gradients in the reactor ranging from as high as 11.54 to as low as 2.3 the highest colony forming units were identified in sections of the soil matrix whose pH was between 9 and 6 and these were areas between 40 mm and 120 mm where the pH is close to 7. The bacteria were able to move into the electrode compartments, but the highest counts were identified in the cathode compartment as compared to the anode compartment in all the experiments (Appendix 1). The difference in the amounts of bacteria in the electrode compartments can be related to the differences in electroosmosis and electrophoresis. The transport mechanism for the bacteria in electrokinetic reactors has been reported to be electroosmosis and electrophoresis. (Kim et al., 2010, Mena et al., 2016). Therefore, the bacteria in the electrode compartments depended on electrophoresis and the electroosmotic flow. But because of the very high pH in the cathode compartment and very low pH in the anode compartment, the bacterial growth was affected as seen by the uneven trend in Figure A1.

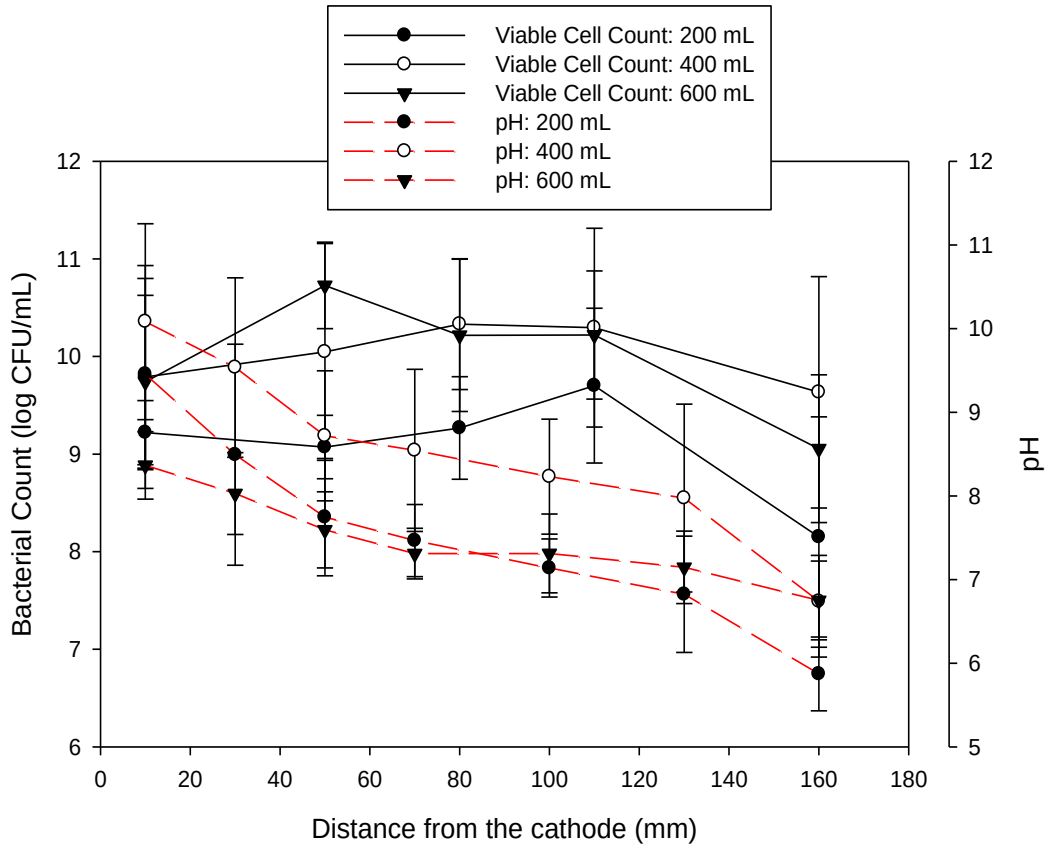


Figure 7. 6. PH and bacterial count variations in different sections along the normalized distance from the cathode

7.7 Hydrocarbon degradation

Das and Chandran (2011) claim that Pseudomonads are the best-known bacteria capable of producing biosurfactants and utilizing hydrocarbons as carbon and energy sources. The degradation of hydrocarbons is however inhibited by low bioavailability of the hydrocarbons to the microorganisms due to their recalcitrant and hydrophobicity properties. Bioavailability is reported to be enhanced by the use of biosurfactants by reducing surface tension and interfacial tension (Batista et al., 2006). In the experiment with 28 g/L and 56 g/L of biosurfactants, the average total organic carbon left after 240 hours was not as low as in the experiment with 86 g/L of biosurfactant (Figure 7.7). The lowest carbon content was registered in 86 g/L as compared to the other biosurfactant concentrations. The average carbon content was 0.1142-, 0.12696-, and 0.0405 mg of carbon/mg

of soil for 28 g/L, 56 g/L and 84 g/L respectively. These were great carbon reductions from the initial total carbon of 0.25 mg of carbon/ mg of soil. These differences in total carbon are due to the variations in degradation which are also due to the variations in biosurfactant concentrations. The lowest biosurfactant concentrations of 28 g/L did not stimulate bacterial growth as much as 56 g/L and 86 g/L which is as a result of the increase in bioavailability of the hydrocarbons to the microbes (Figure 7.5 and 7.6). The total organic carbon concentration in 56 g/L was the highest but the bacterial growth (Figure 7.6) was equally high. This is because of the lower microbial accounts experienced in the initial 100 hours of the experiment with 56 g/L as seen in Figure 7.6. In these 100 hours there was lower degradation of the hydrocarbons as compared to the other two experiments were microbial growth was high in the first 100 hours of the experiment. The gradual reduction in the bacterial counts in 56 g/L and 84 g/L is due to the reduction in the carbon source (oil) towards the end of the experiment. High bioavailability was provided by the higher concentration of biosurfactants at 86 g/L which led to the highest growth of the bacteria in the first 100 hours as compared to the other two biosurfactant concentrations. Most of the hydrocarbons were utilised in this time range and from there on bacterial counts reduced. It's from this that the highest degradation of the hydrocarbons in this study was observed in 84 g/L of biosurfactants as compared to 28 g/L and 56 g/L with the hydrocarbon content in sections 50-100 mm and 100-160.5 mm approaching zero. In all experiments however, the highest degradation was observed between 50 mm and 100 mm normalised distance from the cathode. This comes with no surprise as the highest bacterial counts were also observed between 40 mm and 120 mm normalised distance from the cathode in all experiments (Figure 7.6**)**.

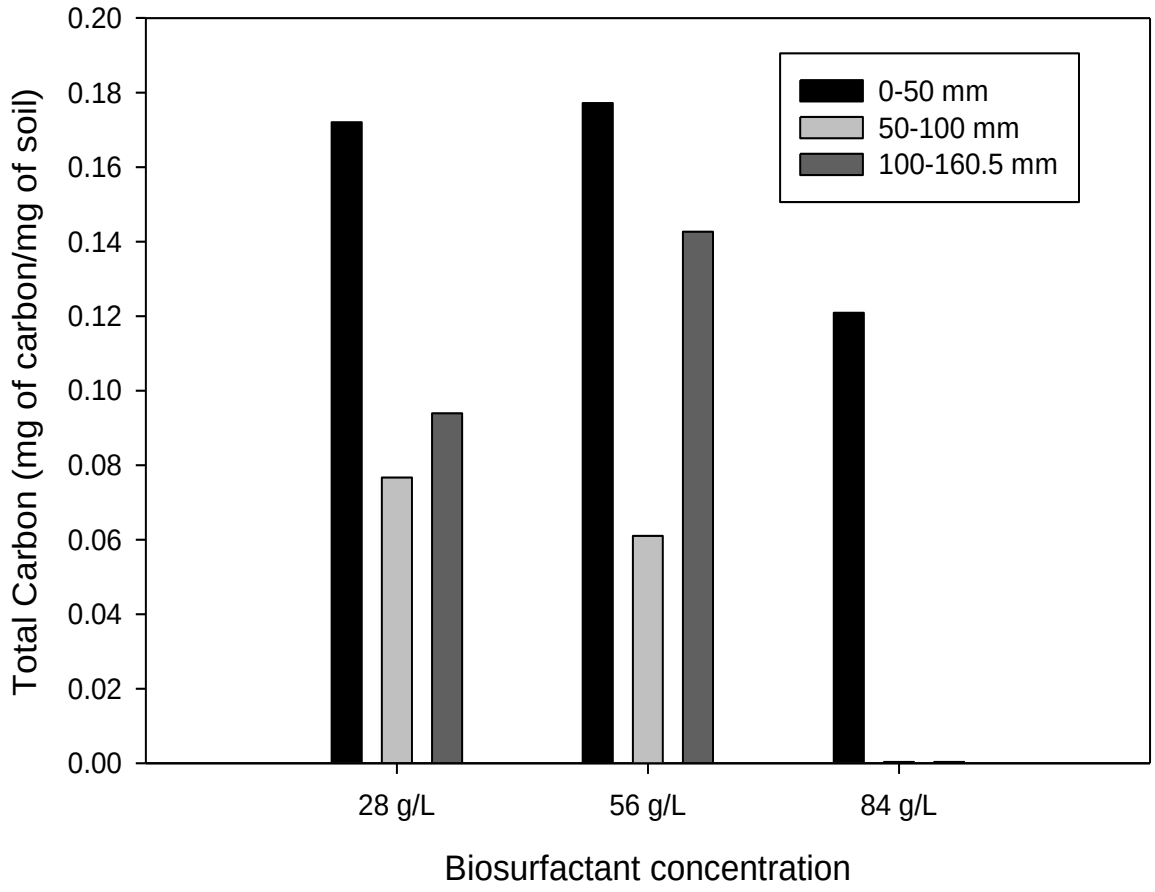


Figure 7. 7. Total carbon left in different sections of the reactor between 0-50 mm, 50-100 mm and 100-160.5 mm from the cathode.

Conclusion

The increase in biosurfactant concentration has a significant effect on oil recovery especially if the shift is from a very low concentration to a higher concentration. The hyper increase in biosurfactant concentration such as three-fold increase is not required for an efficient bioelectrokinetic remediation process calling for a need to identify a threshold value before biosurfactants are applied. Biosurfactants can increase the bioavailability of the hydrocarbon contaminants to the microbes in an electrokinetic reactor justified by the low carbon contents in the soil after the experiments.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

Biosurfactants have the capacity to accelerate oil recovery in the electrokinetic process but the system is mostly affected by the voltage gradient since the highest voltage had the highest oil volume recovered. EOF of oil is possible but is highly affected by the filter pores and oil viscosity. The survival and growth of bacteria under the electric field applied gives promising results for in-situ biosurfactant production.

Approaching electrodes or conventional reduction in electrode spacing has the capacity to increase and maintain high current flow, electroosmotic flow and stabilize pH which may favor oil recovery, bioelectrokinetic remediation as compared to fixed electrodes. The lowest electrode spacing is however most likely to improve the electrokinetic remediation efficiency as compared to larger electrode spacings.

The higher the current the higher the Electroosmotic flow which in turn affects the movement of oil to one common compartment. The addition of biosurfactants to the system does not create a substantive change in the concentration of ionic species in the system considering the contaminated matrix has high concentrations of ionic species. The aggregation of biosurfactants in the electrode compartment leads to recovery of these surface-active substances than can be reused for another bioelectrokinetic process.

The movement of the biosurfactants also means that the impact of biosurfactants in increasing demulsification, bioavailability and coalescence is highest at the beginning of the experiment before they migrate to the appositively charged electrode. The movement of the biosurfactants in the matrix due to electromigration enables the biosurfactants to reach all the contaminant-matrix

interfaces that would otherwise not be reached without biosurfactant motion since the reactor has no shacking or mixing mechanisms.

Much as the ever-inconstant pH affects microbial growth which then affects the biodegradation of the contaminant, it has inconsequential effects to the surface charge of the biosurfactants considering the zeta potential of the biosurfactants remained significantly negative at all pH denominations of alkalinity, neutrality, and acidity.

The increase in biosurfactant concentration has a significant effect on oil recovery especially if the shift is from a very low concentration to a higher concentration. The hyper increase in biosurfactant concentration is however not required for an efficient oil recovery process calling for a need to identify a threshold value before biosurfactants are applied.

The biosurfactants can increase the bioavailability of the hydrocarbon contaminants to the microbes which lead to increased degradation of recalcitrant hydrocarbon contaminants. Future studies should focus on the production of biosurfactants in the reactor followed up by kinetic suggestions of the processes in the bioelectrokinetic reactor.

Regarding real life applications, such a technology would be best fitted to recover oil from oil sludge after studies to recover oil from sludge have been done. This is because such a reactor can be constructed as part of the refinery where biosurfactants are produced in the reactor storing oil waste or sludge. Recommendations regarding future in situ biosurfactant production have been made. Much it is more difficult to apply on site in oil contaminated soil, following studies that have been tried in field scale applications by organisations companies/technologies like the US Army Environmental Agency, Electrosorb^{TM} and Electro-Klean^{TM} electrical separation, for removal of metals and other contaminants. In these field scale applications electrodes are placed in boreholes

in the soil and direct current is applied. The contaminants can then move through the pore water to the electrode, where they could be trapped in the electrode polymer matrix. Studies must be however be done on field scale to ascertain the possibilities of remediation according to the type of soil, and the contaminant.

APPENDICES

Appendix 1: Bacterial counts in the electrode compartments form the beginning to the end of the experiment.

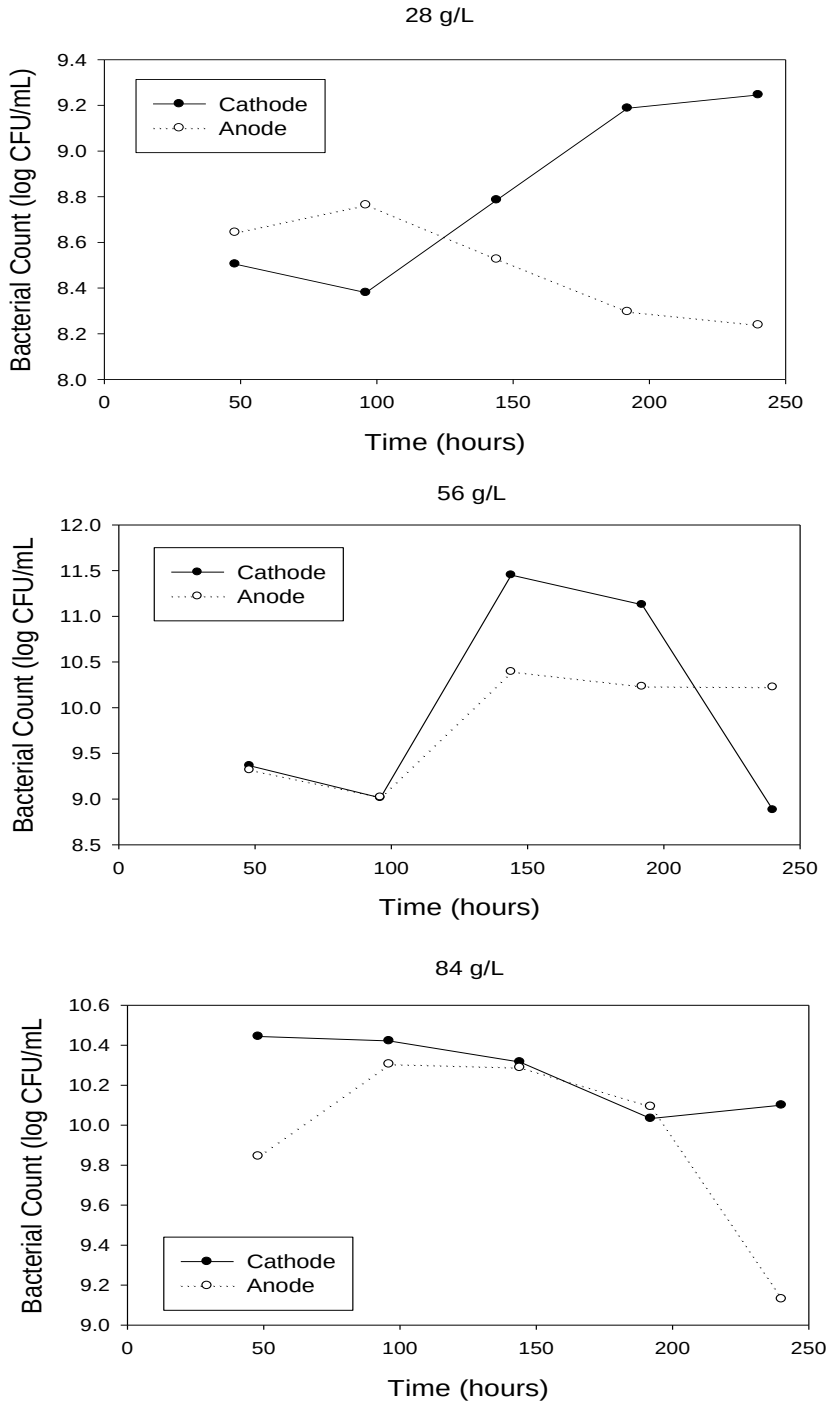


Figure A. 1. Bacteria counts determined in the cathode and anode compartments as a function of time

REFERENCES

Abalos, A., Viñas, M., Sabaté, J., Manresa, M. A., & Solanas, A. M., 2004. Enhanced biodegradation of Casablanca crude oil by a microbial consortium in presence of a rhamnolipid produced by Pseudomonas aeruginosa AT10. *Biodegradation,* 15**,** 249-260.

Acar, Y. B., Galeb, R. J., Alshawabkeh, A. N., Marks, R. E., Puppala, W., Bricka, M. & Parkere, R., 1995. Electrokinetic remediation basics and technology status. *Journal of Hazardous Materials,* 40**,** 117-137.

Agarwal, A. & Liu, Y., 2015. Remediation technologies for oil-contaminated sediments. *Marine Pollution Bulletin,* 101**,** 483-90.

Agnew, K., Cundy, A. B., Hopkinson, L., Croudace, I. W., Warwick, P. E. & Purdie, P., 2011. Electrokinetic remediation of plutonium-contaminated nuclear site wastes: results from a pilot-scale on-site trial. *Journal of Hazardous Materials,* 186**,** 1405-1414.

Al-qahtani, M. R. A., 2011. Effect of oil refinery sludge on plant growth and soil properties. *Journal of Environmental Sciences*, 5(2), 187-193.

Alcantara, M. T., Gomez, J., Pazos, M. & Sanroman, M. A., 2010. Electrokinetic remediation of PAH mixtures from kaolin. *Journal of Hazardous Materials*, 179**,** 1156-1160.

Ali, M. F. & Alqam, M. H., 2000. The role of asphaltenes, resins and other solids in the stabilization of water in oil emulsions and its effects on oil production in Saudi oil fields. *Fuel,* 79, 1309–1316.

Altin, A. & Degirmenci, M., 2005. Lead (II) removal from natural soils by enhanced electrokinetic remediation. *Science of the Total Environment,* 337**,** 1-10.

APHA 2005. In: Eaton A.D., Clesceri L.S., Rice E.W., Greenberg A.E., Franson M.A.H. (Eds.). Standard Methods for the Examination of Water and Wastewater, 25th Edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C., USA.

Asia, I. O., Enweani, I. B. & Eguavoen, I. O., 2006. Characterization and treatment of sludge from the petroleum industry. *African Journal of Biotechnology*, (5), 461-466.

Atlas, R. M., 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological Reviews*, 45(1)**,** 180-209.

Baek, K., Kim, D. H., Park, S. W., Ryu, B. G., Bajargal, T. & Yang, J. S., 2009. Electrolyte conditioning-enhanced electrokinetic remediation of arsenic-contaminated mine tailing. *Journal of Hazardous Materials*, 161**,** 457-462.

Batista, S. B., Mounteer, A. H., Amorim, F. R. & Totola, M. R., 2006. Isolation and characterization of biosurfactant/bioemulsifier-producing bacteria from petroleum contaminated sites. *Bioresource Technology*, 97**,** 868-875.

Bezza, F. A. & Chirwa, E. M. N., 2015. Biosurfactant from Paenibacillus dendritiformis and its application in assisting polycyclic aromatic hydrocarbon (PAH) and motor oil sludge removal from contaminated soil and sand media. *Process Safety and Environmental Protection*, 98**,** 354-364.

Bezza, F. A. & Chirwa, E. M. N., 2017. Possible use of biosurfactant produced by microbial consortium from contaminated soil for microbially enhanced oil recovery. *Chemical Engineering Transactions*, 57, 1411-1416.

Bodour, A. A. & Miller-maier, R. M., 1998. Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *Journal of Microbiological Methods, 32*, 273–280.

Boulakradeche, M. O., Akretche, D. E., Cameselle, C. & Hamidi, N., 2015. Enhanced electrokinetic remediation of hydrophobic organics contaminated soils by the combination of non-ionic and ionic surfactants. *Electrochimica Acta*, 174**,** 1057-1066.

Calvo, C., Silva-castro, G. A., Uad, I., Fandino, G. C., Laguna, J. & Gonzalez-lopez, J., 2008. Efficiency of the EPS emulsifier produced by Ochrobactrum anthropi in different hydrocarbon bioremediation assays. *Journal of Industrial Microbiology & Biotechnology*, 35**,** 1493-1501.

Câmara, J. M. D. A., Sousa, M. A. S. B., Baros Neto, E. L. & Oliveira, M. C. A., 2019. Application of rhamnolipid biosurfactant produced by Pseudomonas aeruginosa in microbial-enhanced oil recovery (MEOR). *Journal of Petroleum Exploration and Production Technology*.

Cameotra, S. S. & Singh, P., 2008. Bioremediation of oil sludge using crude biosurfactants. *International Biodeterioration & Biodegradation*, 62**,** 274-280.

Cameselle C., Gouveia S., Eddine D., Belhadj B., 2013. Advances in electrokinetic remediation for the removal of organic contaminants in soils, e-book, accessed 06.11.2018, DOI: 10.5772/54334.

Cang, L., Fan, G. P., Zhou, D. M. & Wang, Q. Y., 2013. Enhanced-electrokinetic remediation of copper-pyrene co-contaminated soil with different oxidants and pH control. *Chemosphere*, 90**,** 2326-2331.

Caravaca, F. & Roldán, A., 2003. Assessing changes in physical and biological properties in a soil contaminated by oil sludges under semiarid Mediterranean conditions. *Geoderma*, 117**,** 53-61.

Chauhan, A., Fazlurrahman, Oakeshott, J. G. & Jain, R. K., 2008. Bacterial metabolism of polycyclic aromatic hydrocarbons strategies for bioremediation. *Indian Journal of Medical Microbiology*, 48, 95-113.

Coutinho, J. O., Silva, M. P., Moraes, P. M., Monteiro, A. S., Barcelos, J. C., Siqueira, E. P. & Santos, V. L., 2013. Demulsifying properties of extracellular products and cells of Pseudomonas aeruginosa MSJ isolated from petroleum-contaminated soil. *Bioresource Technology,* 128**,** 646-654.

Das, K. & Mukherjee, A. K., 2007. Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresource Technoogyl,* 98**,** 1339-1345.

Das, N. & Chandran, P., 2011. Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnology Research International,* 2011**,** 13.

Dastgheib, S. M., Amoozegar, M. A., Elahi, E., Asad, S. & Banat, I. M., 2008. Bioemulsifier production by a halothermophilic Bacillus strain with potential applications in microbially enhanced oil recovery. *Biotechnology Letters,* 30**,** 263-270.

De Battisti, A. & Ferro, S., 2007. Electrokinetic remediation; Methods of remediation of soils and ground waters. *Electrochimica Acta,* 52**,** 3345-3348.

Desai, J. D. & Banat, I. M., 1997. Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews,* 61(1)**,** 47–64.

Egazar'yants, S. V., Vinokurov, V. A., Vutolkina, A. V., Talanova, M. Y., Frolov, V. I. & Karakhanov, E. A., 2015. Oil Sludge Treatment Processes. *Chemistry and Technology of Fuels and Oils,* 51**,** 506-515.

Electorowicz, M. & Hatim, J., 2000. Application of surfactant enhanced electrokinetics for hydrocarbon contaminated soils. *53rd Canadian Geotechnical Conference.* Montreal.

Elektorowicz, M. & Habibi, S., 2005. Sustainable waste management recovery of fuels from petroleum sludge. *Canadian Journal of Civil Engineering*, 32(1), 164.

Elektorowicz, M., Habibi, S. & Chifrina, R., 2006. Effect of electrical potential on the electro-demulsification of oily sludge. *Journal of Colloid and Interface Science,* 295**,** 535-541.

Epelde, L., Hernandez-allica, J., Becerril, J. M., Blanco, F. & Garbisu, C., 2008. Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. *Science of the Total Environment,* 401**,** 21-28.

Fadhile Almansoory, A., Abu hasan, H., Idris, M., Sheikh Abdullah, S. R., Anuar, N. & Musa Tibin, E. M., 2017. Biosurfactant production by the hydrocarbon-degrading bacteria (HDB) Serratia marcescens: Optimization using central composite design (CCD). *Journal of Industrial and Engineering Chemistry,* 47**,** 272-280.

Fadzil, S. M., Sarmani, S., Majid, A. A., Khoo, K. S. & Hamzah, A., 2010. k 0-INAA measurement of levels of toxic elements in oil sludge and their leachability. *Journal of Radioanalytical and Nuclear Chemistry*, 287**,** 41-47.

García-rubio, A., Rodríguez-Maroto, J. M., Gómez-Lahoz, C., García-Herruzo, F. & Vereda-Alonso, C., 2011. Electrokinetic remediation: The use of mercury speciation for feasibility studies applied to a contaminated soil from Almadén. *Electrochimica Acta,* 56**,** 9303-9310.

George, S. & Jayachandran, K., 2013. Production and characterization of rhamnolipid biosurfactant from waste frying coconut oil using a novel Pseudomonas aeruginosa D. *Journal of Applied Microbiology*, 114**,** 373-383.

Giannis, A., Gidarakos, E. & Skouta, A., 2007. Application of sodium dodecyl sulfate and humic acid as surfactants on electrokinetic remediation of cadmium-contaminated soil. *Desalination*, 211**,** 249-260.

Giannis, A., Pentari, D., Wang, J. Y. & Gidarakos, E., 2010. Application of sequential extraction analysis to electrokinetic remediation of cadmium, nickel and zinc from contaminated soils. *Journal of Hazardous Materials* 184**,** 547-554.

Gomes, H. I., Dias-ferreira, C. & Ribeiro, A. B., 2012. Electrokinetic remediation of organochlorines in soil: enhancement techniques and integration with other remediation technologies. *Chemosphere*, 87**,** 1077-1090.

Gopang, I. A., Mahar, H., Jatoi, A. S., Akhtar, K. S., Omer, M. & Azeem, M. S. 2016. Characterization of the sludge deposits in crude oil. *Journal of Faculty of Engineering & Technology*, 23(1), 57-64.

Gudiña, E. J., Pereira, J. F. B., Rodrigues, L. R., Coutinho, J. A. P. & Teixeira, J. A. 2012. Isolation and study of microorganisms from oil samples for application in Microbial Enhanced Oil Recovery. *International Biodeterioration & Biodegradation*, 68**,** 56-64.

Gudina, E. J., Rodrigues, A. I., Alves, E., Domingues, M. R., Teixeira, J. A. & Rodrigues, L. R., 2015. Bioconversion of agro-industrial by-products in rhamnolipids toward applications in enhanced oil recovery and bioremediation. *Bioresource Technology*, 177**,** 87-93.

Guedes, P., Mateus, E. P., Couto, N., Rodriguez, Y. & Ribeiro, A. B., 2014. Electrokinetic remediation of six emerging organic contaminants from soil. *Chemosphere*, 117**,** 124-31.

Hajivand, P. & Vaziri, A., 2015. Optimization of demulsifier formulation for separation of water from crude oil emulsions. *Brazilian Journal of Chemical Engineering*, 32**,** 107-118.

Hassanshahian, M., Emtiazi, G. & Cappello, S., 2012. Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. *Marine Pollution Bulletin*, 64**,** 7-12.

Hu, G., Li, J. & Zeng, G., 2013. Recent development in the treatment of oily sludge from petroleum industry: a review. *Journal of Hazardous Materials*, 261**,** 470-490.

Huang, X. F., Liu, J., Lu, L. J., Wen, Y., Xu, J. C., Yang, D. H. & Zhou, Q. 2009. Evaluation of screening methods for demulsifying bacteria and characterization of lipopeptide bio-demulsifier produced by Alcaligenes sp. *Bioresource Technology*, 100**,** 1358-1365.

INSPQ., 2015. Public health issues related to gas and oil hydrocarbon exploration and production. Environmental Health and Toxicology Branch, Biological Risks and Occupational Health Branch, Quebec, viewed 10 May 2018, <https://www.inspq.qc.ca/en/publications/latest>.

Islam, B., 2015. Petroleum sludge, its treatment and disposal: A review. *International Journal of Chemical Sciences*, 13(4), 1584-1602.

Jeon, E. K., Jung, J. M., Kim, W. S., Ko, S. H. & Baek, K., 2015. In situ electrokinetic remediation of As-, Cu-, and Pb-contaminated paddy soil using hexagonal electrode configuration: a full-scale study. *Environmental Science and Pollution Research*, 22**,** 711-720.

Kim, D. H., Jeon, C. S., Baek, K., Ko, S. H. & Yang, J. S., 2009a. Electrokinetic remediation of fluorine-contaminated soil: conditioning of anolyte. *Journal of Hazardous Materials*, 161**,** 565-569.

Kim, D. H., Ryu, B. G., Park, S. W., Seo, C. I. & Baek, K., 2009b. Electrokinetic remediation of Zn and Ni-contaminated soil. *Journal of Hazardous Materials*, 165**,** 501-505.

Kim, D. H., Yoo, J. C., Hwang, B. R., Yang, J. S. & Baek, K. 2014a. Environmental assessment on electrokinetic remediation of multimetal-contaminated site: a case study. *Environmental Science and Pollution Research*, 21**,** 6751-6758.

Kim, S. H., Han, H. Y., Lee, Y. J., Kim, C. W. & Yang, J. W., 2010. Effect of electrokinetic remediation on indigenous microbial activity and community within diesel contaminated soil. *Science of the Total Environment*, 408**,** 3162-3168.

Kim, W. S., Jeon, E. K., Jung, J. M., Jung, H. B., Ko, S. H., Seo, C. I. & Baek, K. 2014b. Field application of electrokinetic remediation for multi-metal contaminated paddy soil using two-dimensional electrode configuration. *Environmental Science and Pollution Research*, 21**,** 4482-4491.

Kimura, T., Takase, K. & Tanaka, S., 2007a. Concentration of copper and a copper-EDTA complex at the pH junction formed in soil by an electrokinetic remediation process. *Journal of Hazardous Materials*, 143**,** 668-672.

Kimura, T., Takase, K., Terui, N. & Tanaka, S., 2007b. Ferritization treatment of copper in soil by electrokinetic remediation. *Journal of Hazardous Materials*, 143**,** 662-667.

Kingston, P. F., 2002. Long-term environmental impact of oil spills. *Spill Science & Technology Bulletin*, 7(1–2)**,** 53–61.

Kisic, I., Mesic, S., Basic, F., Brkic, V., Mesic, M., Durn, G., Zgorelec, Z. & Bertovic, L., 2009. The effect of drilling fluids and crude oil on some chemical characteristics of soil and crops. *Geoderma*, 149**,** 209-216.

Kornilovich, B., Mishchuk, N., Abbruzzese, K., Pshinko, G. & Klishchenko, R., 2005. Enhanced electrokinetic remediation of metals-contaminated clay. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 265**,** 114-123.

Kriipsalu, M., Marques, M. & Maastik, A., 2008. Characterization of oily sludge from a wastewater treatment plant flocculation-flotation unit in a petroleum refinery and its treatment implications. *Journal of Material Cycles and Waste Management,* 10**,** 79-86.

Kwon, T. S. & Lee, J. Y., 2015. Options for reducing oil content of sludge from a petroleum wastewater treatment plant. *Waste Management & Research*, 33**,** 937-940.

Langangen, O., Olsen, E., Stige, L. C., Ohlberger, J., Yaragina, N. A., Vikebo, F. B., Bogstad, B., Stenseth, N. C. & Hjermann, D. O. 2017. The effects of oil spills on marine fish: Implications of spatial variation in natural mortality. *Marine Pollution Bulletin*, 119**,** 102-109.

Leahy, J. G. & Colwell, R. R., 1990. Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews,* 54(3)**,** 305-315.

Lear, G., Harbottle, M. J., Sills, G., Knowles, C. J., Semple, K. T. & Thompson, I. P., 2007. Impact of electrokinetic remediation on microbial communities within PCP contaminated soil. *Environmental Pollution*, 146**,** 139-46.

Li, D., Tan, X.-Y., WU, X.-D., Pan, C. & Xu, P., 2014. Effects of electrolyte characteristics on soil conductivity and current in electrokinetic remediation of lead-contaminated soil. *Separation and Purification Technology,* 135**,** 14-21.

Li, G., Guo, S., Li, S., Zhang, L. & Wang, S., 2012. Comparison of approaching and fixed anodes for avoiding the ‘focusing’ effect during electrokinetic remediation of chromium-contaminated soil. *Chemical Engineering Journal,* 203**,** 231-238.

Lim, M. W., Lau, E. V. & Poh, P. E., 2016. A comprehensive guide of remediation technologies for oil contaminated soil - Present works and future directions. *Marine Pollution Bulletin,* 109**,** 14-45.

Lima, T. M., Procopio, L. C., Brandao, F. D., Leao, B. A., Totola, M. R. & Borges, A. C., 2011. Evaluation of bacterial surfactant toxicity towards petroleum degrading microorganisms. *Bioresource Technology*, 102**,** 2957-2964.

Liu, W., Wang, X., Wu, L., Chen, M., Tu, C., Luo, Y. & Christie, P., 2012. Isolation, identification and characterization of Bacillus amyloliquefaciens BZ-6, a bacterial isolate for enhancing oil recovery from oily sludge. *Chemosphere*, 87**,** 1105-1110.

López-Vizcaíno, R., Yustres, A., León, M. J., Saez, C., Cañizares, P., Rodrigo, M. A. & Navarro, V., 2017. Multiphysics Implementation of Electrokinetic Remediation Models for Natural Soils and Porewaters. *Electrochimica Acta*, 225**,** 93-104.

Loya, Y. & Rinkevich, B., 1980. Effects of oil pollution on coral reef communities. *Inter-Research Science Center*, 3, 167-180.

Lu, P., Feng, Q., Meng, Q. & Yuan, T., 2012. Electrokinetic remediation of chromium- and cadmium-contaminated soil from abandoned industrial site. *Separation and Purification Technology*, 98**,** 216-220.

Makkar, R. S. & Rockne, K. J., 2003. Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry*, 22**,** 2280–2292.

Masi, M., Ceccarini, A. & Iannelli, R., 2017. Multispecies reactive transport modelling of electrokinetic remediation of harbour sediments. *Journal of Hazardous Materials*, 326**,** 187-196.

Maturi, K. & Reddy, K. R., 2008. Cosolvent-enhanced desorption and transport of heavy metals and organic contaminants in soils during electrokinetic remediation. *Water Air and Soil Pollution*, 189**,** 199-211.

Mena, E., Villaseñor, J., Rodrigo, M. A. & Cañizares, P., 2016. Electrokinetic remediation of soil polluted with insoluble organics using biological permeable reactive barriers: Effect of periodic polarity reversal and voltage gradient. *Chemical Engineering Journal*, 299**,** 30-36.

Morikawa, M., Hirata, Y. & Imanaka, T., 2000. A study on the structure–function relationship of lipopeptide biosurfactants. *Biochimica et Biophysica Acta*, 1488(3)**,** 211–218.

Mukherjee, S., Das, P. & Sen, R., 2006. Towards commercial production of microbial surfactants. *Trends in Biotechnology*, 24**,** 509-515.

Mulligan, C. N., 2009. Recent advances in the environmental applications of biosurfactants. *Current Opinion in Colloid & Interface Science*, 14**,** 372-378.

Mulligan, C. N., Yong, R. N. & Gibbs, B. F., 2001. Surfactant-enhanced remediation of contaminated soil: a review. *Engineering Geology*, 60**,** 371-380.

Narong, P. & James, A. E., 2006. Effect of pH on the ζ-potential and turbidity of yeast suspensions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 274**,** 130-137.

Nelson-Smith, A., 1968. The Effects of Oil Pollution and Emulsifier Cleansing on Shore Life in South-West Britain. *Journal of Applied Ecology*, 5(1), 97-107.

Noparat, P., Maneerat, S. & SaimmaI, A., 2014. Utilization of palm oil decanter cake as a novel substrate for biosurfactant production from a new and promising strain of Ochrobactrum anthropi 2/3. *World Journal of Microbiology and Biotechnology*, 30**,** 865-877.

Ouhadi, V. R., Yong, R. N., Shariatmadari, N., Saeidijam, S., Goodarzi, A. R. & Safari-Zanjani, M., 2010. Impact of carbonate on the efficiency of heavy metal removal from kaolinite soil by the electrokinetic soil remediation method. *Journal of Hazardous Materials*, 173**,** 87-94.

Park, S. W., Lee, J. Y., Yang, J. S., Kim, K. J. & Baek, K., 2009. Electrokinetic remediation of contaminated soil with waste-lubricant oils and zinc. *Journal of Hazardous Materials,* 169**,** 1168-1172.

Pazoki, M. & Hasanidarabadi, B., 2017. Management of toxic and hazardous contents of oil sludge in Siri Island. *Global Journal of Environmental Science and Management,* 3(1), 33-42.

Pazos, M., Sanroman, M. A. & Cameselle, C., 2006. Improvement in electrokinetic remediation of heavy metal spiked kaolin with the polarity exchange technique. *Chemosphere,* 62**,** 817-822.

Peng, G., Tian, G., Liu, J., Bao, Q. & Zang, L., 2011. Removal of heavy metals from sewage sludge with a combination of bioleaching and electrokinetic remediation technology. *Desalination,* 271**,** 100-104.

Pereira, J. F. B., Gudiña, E. J., Costa, R., Vitorino, R., Teixeira, J. A., Coutinho, J. A. P. & Rodrigues, L. R., 2013. Optimization and characterization of biosurfactant production by Bacillus subtilis isolates towards microbial enhanced oil recovery applications. *Fuel,* 111**,** 259-268.

Pezeshki, S. R., Hester, M. W., Lin, Q. & Nyman, J. A., 2000. The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: a review *Environmental Pollution***,** 108, 129–139.

Pham, T. D., Shrestha, R. A., Virkutyte, J. & Sillanpää, M., 2009. Combined ultrasonication and electrokinetic remediation for persistent organic removal from contaminated kaolin. *Electrochimica Acta,* 54**,** 1403-1407.

Popov, K., Glazkova, I., Yachmenev, V. & Nikolayev, A. 2008. Electrokinetic remediation of concrete: effect of chelating agents. *Environmental Pollution*, 153**,** 22-28.

Prince, R. C., Lessard, R. R. & Clark, J. R., 2003. Bioremediation of Marine Oil Spills. *Oil & Gas Science and Technology*, 58(4), 463-468.

Reddy, K. R. & Saichek, R. E., 2004. Enhanced Electrokinetic Removal of Phenanthrene from Clay Soil by Periodic Electric Potential Application. *Journal of Environmental Science and Health, Part A*, 39**,** 1189-1212.

Reddy, K. R. & Shirani, A. B., 1996. Electrokinetic remediation of metal contaminated glacial tills. *Geotechnical and Geological Engineering*, 15**,** 3-29.

Rikalovic, M., Gojgic-Cvijovic, G., Vrvic, M. & Karadzic, I., 2012. Production and characterization of rhamnolipids from Pseudomonas aeruginosa san ai. *Journal of the Serbian Chemical Society*, 77**,** 27-42.

Rocha, E. S. F. C. P., Roque, B. A. C., Rocha, E. S. N. M. P., Rufino, R. D., Luna, J. M., Santos, V. A., Banat, I. M. & Sarubbo, L. A., 2017. Yeasts and bacterial biosurfactants as demulsifiers for petroleum derivative in seawater emulsions. *AMB Express*, 7**,** 202.

Rodrigues, L., Moldes, A., Teixeira, J. & Oliveira, R. 2006. Kinetic study of fermentative biosurfactant production by Lactobacillus strains. *Biochemical Engineering Journal*, 28**,** 109-116.

Ron, E. Z. & Rosenberg, E., 2002. Biosurfactants and oil bioremediation. *Current Opinion in Biotechnology*, 13**,** 249-252.

Roulier, M., Kemper, M., Al-Abed, S., Murdoch, L., Cluxton, P., Chen, J. L. & Davis-Hoover, W., 2000. Feasibility of electrokinetic soil remediation in horizontal Lasagna cells. *Journal of Hazardous Materials*, B77**,** 161-176.

Rozas, F. & Castellote, M., 2012. Electrokinetic remediation of dredged sediments polluted with heavy metals with different enhancing electrolytes. *Electrochimica Acta*, 86**,** 102-109.

Rutigliano, L., Fino, D., Saracco, G., Specchia, V. & Spinelli, P. 2008. Electrokinetic remediation of soils contaminated with heavy metals. *Journal of Applied Electrochemistry*, 38**,** 1035-1041.

Sawada, A., Mori, K. I., Tanaka, S., Fukushima, M. & Tatsumi, K., 2004. Removal of Cr (VI) from contaminated soil by electrokinetic remediation. *Waste Management*, 24**,** 483-490.

Schmidt, C. A., Barbosa, M. C. & De Almeida Mde, S., 2007. A laboratory feasibility study on electrokinetic injection of nutrients on an organic, tropical, clayey soil. *Journal of Hazardous Materials*, 143**,** 655-661.

Shen, Z., Chen, X., Jia, J., Qu, L. & Wang, W., 2007. Comparison of electrokinetic soil remediation methods using one fixed anode and approaching anodes. *Environmental Pollution*, 150**,** 193-199.

Shu, J., Liu, R., Liu, Z., Du, J. & Tao, C., 2015. Electrokinetic remediation of manganese and ammonia nitrogen from electrolytic manganese residue. *Environmental Science and Pollution Research*, 22**,** 16004-16013.

Singh, P. & Cameotra, S. S., 2004. Enhancement of metal bioremediation by use of microbial surfactants. *Biochemical and Biophysical Research Communications*, 319**,** 291-297.

Souza, E. C., Vessoni-penna, T. C. & De Souza Oliveira, R. P., 2014. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. *International Biodeterioration & Biodegradation*, 89**,** 88-94.

Sriram, M. I., Gayathiri, S., Gnanaselvi, U., Jenifer, P. S., Mohan Raj, S. & Gurunathan, S., 2011. Novel lipopeptide biosurfactant produced by hydrocarbon degrading and heavy metal tolerant bacterium Escherichia fergusonii KLU01 as a potential tool for bioremediation. *Bioresource Technology*, 102**,** 9291-9295.

Trummler, K., Effenberger, F. & Syldatk, C., 2003. An integrated microbial/enzymatic process for production of rhamnolipids and L-(+)-rhamnose from rapeseed oil with Pseudomonas sp. DSM 2874. *European Journal of Lipid Science and Technology*, 105**,** 563–571.

Tyagi, M., Da Fonseca, M. M. & De Carvalho, C. C., 2011. Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation*, 22**,** 231-241.

U.S.EPA., 1997. Resource guide for electrokinetics laboratory and field processes applicable to radioactive and hazardous mixed wastes in soil and groundwater from 1992 to 1997. *In:* U.S.EPA (ed.) *Air and Radiation.*

UK. Enviromental Agency., 2007. How to find out if waste oil and wastes that contain oil are hazardous; A guide to the Hazardous Waste Regulations.Accessed 10 September 2018, <www.environment-agency.gov.uk>.

UNEP., 2014. Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal. Accessed 10 September 2018, <https://www.basel.int/Portals/4/Basel%20Convention/docs/text/BaselConventionText-e.pdf>.

Uzoigwe, C., Burgess, J. G., Ennis, C. J. & Rahman, P. K. 2015. Bioemulsifiers are not biosurfactants and require different screening approaches. *Frontiers in Microbiology*, 6**,** 245.

Virkutytea, J., Sillanpaa, M. & Latostenmaa, P., 2002. Electrokinetic soil remediation-critical overview. *The Science of the Total Environment*, 289**,** 97-121.

Vocciante, M., Caretta, A., Bua, L., Bagatin, R. & Ferro, S., 2016. Enhancements in electroKinetic remediation technology: Environmental assessment in

comparison with other configurations and consolidated solutions. *Chemical Engineering Journal*, 289**,** 123-134.

Volkering, F., Breure, A. M. & Rulkens, W. H., 1998. Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation*, 8**,** 401-417.

Wang, Q., Fang, X., Bai, B., Liang, X., Shuler, P. J., Goddard, W. A., 3rd & Tang, Y., 2007. Engineering bacteria for production of rhamnolipid as an agent for enhanced oil recovery. *Biotechnology and Bioengineering*, 98**,** 842-853.

Wang, Q. Y., Zhou, D. M., Cang, L., Li, L. Z. & Wang, P., 2009. Solid/solution Cu fractionations/speciation of a Cu contaminated soil after pilot-scale electrokinetic remediation and their relationships with soil microbial and enzyme activities. *Environmental Pollution*, 157**,** 2203-2208.

Wu, M., Dick, W. A., Li, W., Wang, X., Yang, Q., Wang, T., Xu, L., Zhang, M. & Chen, L., 2016. Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil. *International Biodeterioration & Biodegradation*, 107**,** 158-164.

Xu, S., Guo, S., Wu, B., Li, F. & Li, T., 2014. An assessment of the effectiveness and impact of electrokinetic remediation of pyrene contaminated soil. *Journal of Environmental Sciences*, 26, 229 0 – 2297

Xu, Y., Xu, X., Hou, H., Zhang, J., Zhang, D. & Qian, G., 2016. Moisture content-affected electrokinetic remediation of Cr(VI)-contaminated clay by a hydrocalumite barrier. *Environmental Science and Pollution Research*, 23**,** 6517-6523.

Xu, Y., Zhang, C., Zhao, M., Rong, H., Zhang, K. & Chen, Q. 2017. Comparison of bioleaching and electrokinetic remediation processes for removal of heavy metals from wastewater treatment sludge. *Chemosphere*, 168**,** 1152-1157.

Yang, L., Nakhla, G. & Bassi, A., 2005. Electro-kinetic dewatering of oily sludges. *Journal of Hazardous Materials*, 125**,** 130-40.

Yeung, A. T. & Gu, Y. Y., 2011. A review on techniques to enhance electrochemical remediation of contaminated soils. *Journal of Hazardous Materials*, 195**,** 11-29.

Yuan, C. & Chiang, T. S., 2008. Enhancement of electrokinetic remediation of arsenic spiked soil by chemical reagents. *Journal of Hazardous Materials*, 152**,** 309-315.

Yuan, L., Xu, X., Li, H., Wang, N., Guo, N. & Yu, H., 2016. Development of novel assisting agents for the electrokinetic remediation of heavy metal-contaminated kaolin. *Electrochimica Acta*, 218**,** 140-148.

Zahed, M. A., Aziz, H. A., Isa, M. H., Mohajeri, L., Mohajeri, S. & Kutty, S. R., 2011. Kinetic modeling and half life study on bioremediation of crude oil dispersed by Corexit 9500. *Journal of Hazardous Materials*, 185**,** 1027-1031.

Zhang, T., Zou, H., Ji, M., Li, X., Li, L. & Tang, T., 2014. Enhanced electrokinetic remediation of lead-contaminated soil by complexing agents and approaching anodes. *Environmental Science and Pollution Research*, 21**,** 3126-3133.

Zheng, C., Wang, M., Wang, Y. & Huang, Z., 2012. Optimization of biosurfactant-mediated oil extraction from oil sludge. *Bioresource Technology*, 110**,** 338-342.

Zhu, S., Zhou, M. & Zhang, S., 2015. Enhanced electrokinetic remediation of fluorine-contaminated soil by applying an ammonia continuous circulation system. *Korean Journal of Chemical Engineering*, 33**,** 547-552.