REMOVAL OF HEAVY METALS FROM DIESEL-POLLUTED WATER USING *KLEBSIELLA SPECIES* ISOLATED FROM DIESEL-POLLUTED SOIL

BY

IGE OLUMIDE ABAYOMI

B.Sc. (PURE CHEMISTRY)

OLABISI ONABANJO UNIVERSTY

AGO-IWOYE

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc.) IN CHEMICAL ENGINEERING.

FACULTY OF TECHNOLOGY,

OBAFEMI AWOLOWO UNIVERSITY,

ILE-IFE.

2014

OBAFEMI AWOLOWO UNIVERSITY

AUTHORIZATION TO COPY

AUTHOR: IGE Olumide Abayomi

TITLE: Removal of heavy metals from diesel-polluted water using *Klebsiella species* isolated from diesel-polluted soil

DEGREE: M.Sc. (Chemical Engineering)

YEAR: 2014

I**,** IGE Olumide Abayomi hereby authorize the Hezekiah Oluwasanmi Library to copy my thesis in whole or part, in response to request from individual researchers and organizations for the purpose of private study or research.

Signature:……. Date:…….

CERTIFICATION

This is to certify that this research work by IGE Olumide Abayomi (TP11/12/H/1373) of the Department of Chemical Engineering, Obafemi Awolowo University, Ile-Ife was carried out under our supervision.

……. ……

Prof. S.K Layokun **Date**

(Supervisor)

……. …….

Dr. (Mrs) E.F Aransiola **Date**

(Co-Supervisor)

……. …….

Dr. A.S Osunleke **Date**

(Chief Examiner)

DEDICATION

This work is dedicated to the families of Ige, Aina, Adebiyi and all those who have helped me in my journey through life.

ACKNOWLEDGEMENT

My profound gratitude goes to GOD who has been my source of strength for giving me the opportunity to reach this milestone in my academic pursuit.

I am grateful to Prof. S.K. Layokun for his meaningful contributions and his readiness to assist throughout the duration of this work. I appreciate the efforts of my co-supervisor Dr. (Mrs) E.F. Aransiola. I want to say a big thank you to Prof. O. Taiwo, Prof. A.N Anozie, Prof. F.A. Akeredolu, Prof. J.A Sonibare, Dr. A.S Osunleke, Dr. O.J Odejobi, and Dr. E. Betiku for their support during the course of my study. My appreciation goes to Dr. E.A Taiwo for his love and support. I will always have fond memories of you. Special thanks go to Mr Oni and Mrs. Awotipe of the Department of Microbiology for assisting me with the microbial aspect of my work.

My appreciation goes to Mr. Adetayo Adebiyi (You are a diamond in the rough) for his financial support without which I would not have been able to complete this programme. To Dr. O.O Adebiyi, I say a big thank you for being a blessing to me. To Mr Muyiwa Olokun, Mr Samuel Soyombo, Mr. B.A Odunlami, Mrs Bamgbose and Mr Uwaigbe I want to say I love and cherish u all.

Here comes the moment I have been waiting for to appreciate my family who gave me the much needed support when the going was tough - My baby (Olamide Ige), Mrs.Adebisi Taiwo, Mr. Seun Aina and Mr. Abayomi Ige.

To my band of friends Mr B. Okorende, Mr O. Falowo, Mr. F. Adeyeye, Mr H. Emeko, Mr C. Jisieke, Mr M. Oke, Mr M. Oloko-Oba, Mr. O. Olatunde, Mr Aremu Mr Y. Agbontafara, Mr Aremu, Engr. Alhaja, Mr Oladoyinbo, Mr E. Akintunde, Mr. Olalekan Jolayemi, Mr. Oluwaseun Fasanmi, Mr Femi Fagite, and my big bro, Mr Dayo Owoeye, you are all cherished.

Finally, I will like to remember, appreciate and honour Mr Fayomi, Mr Odutola Oresanya, Mr Obafemi Awolowo and Prof. Olumide Sosanwo all of blessed memory for placing my feet on the path of success. I will remember you all till I breathe my last on the surface of the earth.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE **TITLE**

PAGE

ABSTRACT

This project investigated the microorganisms indigenous to a diesel-oil-polluted soil sample, their potential use in bioremediation of heavy metals in diesel-oil-polluted water using ex situ technique and factors affecting bioremediation. This was with a view of reducing the hazards that arise due to heavy metal pollution.

A diesel-oil-polluted soil sample was collected from a mechanic workshop. A working sample was prepared in a conical flask by suspending 0.5 g of the diesel-oil-polluted soil sample in 50 ml of sterile distilled water (dilution of 10^{-2} w/v) in a 100 ml conical flask in which some glass chips were inserted. The flask was carefully agitated to obtain a uniform suspension. Dilutions of 10^{-4} and 10^{-6} were carried out in test tubes A and B. One milliliter of each dilution in tubes A and B was transferred into properly labelled petri dishes respectively. Culturing was done by making use of 20 ml molten sterile Eosin Methylene Blue agar and left to stand for the medium to set. They were incubated at 35 ^{o}C for 48 h. Colonial characteristics of the mixed culture obtained was observed. The differential colonies were isolated by sub-culturing into nutrient agar slants and labelled accordingly for use. The 18 h cultures were gram stained to study the morphological characteristics of the cultures and to ensure their purification. The ability of the isolates to grow on a heavy metal polluted medium was investigated. A digestion process was carried out and the sample was analyzed for Copper, Nickel, Chromium and Cadmium using atomic absorption spectrometry. Two hundred and fifty millilitres of bushnell-haas medium was distributed into each conical flask in which 10 ml of diesel-polluted water was added. Sterilization by autoclaving was done at 121 ^{o}C for 15 min. The medium was inoculated in a sterile environment with pure and mixed cultures of the *Klebsiella species* and labelled appropriately. They were incubated in a New Brunswick Gyratory shaker for 16 days. Samples were withdrawn at four days interval for analysis. Design-Expert 8.0.3.1 was used for the design, modelling and optimization using time and pH as the two variables.

The isolated organisms isolated and used for the bioremediation studies were *Klebsiella edwardsii*, *Klebsiella oxytoca, Klebsiella pneumoniae* and *Pseudomonas cepacia*. When *Klebsiella edwardsii* was used as bioremediating agent, concentration of Cu, Ni, Cr and Cd reduced by 87.28, 81.55, 98.30 and 92.59% respectively. *Klebsiella oxytoca* removed 85.55, 80.58, 98.56 and 96.29% of Cu, Ni, Cr and Cd respectively from the original sample. When the polluted water was treated with *Klebsiella pneumoniae,* percentage reduction in concentration of Cu, Ni, Cr and Cd were 89.59, 83.49, 98.49 and 88.88% respectively. In the mixed culture of *Klebsiella edwardsii*, *Klebsiella oxytoca* and *Klebsiella pneumoniae*, percentage reduction in values were 82.65, 83.49, 98.27 and 92.59% for Cu, Ni, Cr and Cd respectively. In the optimization studies, optimum pH and time for the bioremediation of the heavy metals of interest were 7.46 and 16 days respectively.

This study revealed that *Klebsiella species* is an efficient bioremediating agent for Cu, Ni, Cr and Cd. It also showed that pH and time have a significant effect on bioremediation of heavy metals.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Nigeria is one of the major producers of crude oil in the world and contamination of the environment through its exploitation, refining, transportation and storage has steadily increased. Crude or refined oil and related products enter into land, land waters, estuaries or open ocean through oil well blowouts, tanker accidents, accidental rupture of oil pipelines and spills of used petroleum products. In the Niger Delta Area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment (Korie-Siakpere, 1998; Odiete, 1999**).** All these activities lead to environmental pollution which poses a great threat to terrestrial and aquatic life and causes disruption in the ecosystem. The impact of pollution cannot be underestimated since some hydrocarbon components are carcinogenic in nature (Shukla and Singh, 2012).

It is a common practice in Nigerian cities and towns to allocate large portions of land of about 5 hectares or more, to groups of small scale auto-mechanic businesses and these are designated as villages where they locate their workshops and repair yards to offer their services to the public. Activities conducted in these shops involve working with oils, greases, petrol, diesel, battery electrolyte which may be accidentally spilled unto bare soil (Adelekan and Abegunde, 2011). Diesel oil, which is one of the major products of crude oil, constitutes a source of pollution in our environment (Nwaogu *et al,* 2008). Diesel pollution can occur through leakage from storage tanks, refueling of vehicles, wrecks of oil tankers and improper disposal by diesel tankers and by mechanics (Venosa *et al*, 1996).

Diesel oil pollution also occurs through careless and indiscriminate disposal of used diesel oil in fuel stations and depots. This has led to reduced plant growth on agricultural land. Suggested reasons for the reduced plant growth in diesel oil contaminated soils range from direct toxic effect on plants (Baker, 1982) and reduced germination (Udo and Fayemi, 1975) to unsatisfactory soil condition due to insufficient aeration of the soil because of the displacement of air from the space between the soil particles by diesel oil. Due to these activities, heavy metals in diesel oil can be deposited in the soil and enter the human food web thereby constituting risk to the ecosystem as they tend to bioaccumulate and can be transferred from one food chain to another. Consequently they are discovered in various food chains where the results are usually detrimental to plants, animals and humans alike (Abdu and Muazu, 2007). Diesel oil pollution can also lead to an increase in heavy metals concentration in water which could have harmful effects on humans and the environment. The contaminants are able to infiltrate deep into the layer of underground waters and pollute the groundwater as well as the surface water. As a result, the World Health Organisation has set some specified limits for heavy metals concentration in water (Ileckukwu and Okonkwo, 2012).

Biological treatment is an innovative technology available for heavy metal polluted wastewater. Since microorganisms have developed survival strategies in heavy metal polluted habitats, their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or biosorption can be applied either ex situ or in situ to design economical bioremediation processes (Malik, 2004 ; Lin C, 2005). Cases of diesel oils spills are common in the environment. Therefore this work studied the extent and rate to which *Klebsiella edwardsii*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* isolated from a diesel-oil polluted soil can be used to remove Copper, Nickel, Chromium and Cadmium metals from diesel-oil-polluted water.

1.2 Objectives of the Research Study

The objectives of this research work are:

(i)to isolate, characterize and identify the microorganisms present in a diesel-oil-polluted soil.

(ii)to use the microorganisms isolated from (i) to reduce the heavy metal concentration in diesel- oil-polluted water.

(iii)to check the effects of pH and time on the rate of bioremediation of the heavy metals of interest.

1.3 Scope of Research Study

The overall objective of this research study was to use *Klebsiella edwardsii*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* to bioremediate heavy metals in water that was polluted with diesel fuel. The optimization was carried out using Design-Expert 8.0.3.1. software to generate the experimental runs using Central Composite Rotatable Design by taking into consideration two factors: pH and time.

1.4 Justification

Petroleum is a leading contaminant due to its production, transport, use and disposal (Jagadevan and Mukherji. 2004). According to Baker and Herson (1994), the national priority list of sites polluted by petroleum is about 1,200 with potential sites numbering about 32,000. Bioremediation has proven to be an improved alternative technology to destroy or render harmless various contaminants. This study will provide information on the best conditions for the bioremediation of heavy metals using the *Klebsiella spp*. It will also show that microorganisms in polluted environments can be used for environmentally friendly purposes.