



Bacterial degradation of various lubricant oils and their environmental impact

Thesis

Submitted for partial Fulfillment of Master degree in

Microbiology

BY

Ali Mohamed Ali Saeed

(B.Sc. Microbiology, 2004)

Supervisors

**Prof Dr. Ahmed Osman
Mostafa**

**Prof. of Biochemistry,
Biochemistry Department,
Faculty of science,
Ain Shams University.**

**Dr. Khaled Zakaria El-
Baghdady**

**Lecturer of Microbiology,
Microbiology Department,
Faculty of science,
Ain Shams University.**

Dr. Abeer E. M. Zakaria

**Lecturer of Microbiology,
National Center of Radiation
Research and Technology (NCRRT).**

**AIN SHAMS UNIVERSITY
FACULTY OF SCIENCE
DEPT. OF MICROBIOLOGY**

2010

Prof Dr. Ahmed Osman Mostafa

Prof. of Biochemistry
Biochemistry department,
Faculty of science,
Ain shams University.

Dr. Khaled Zakaria

Lecturer of microbiology
Microbiology department,
Faculty of science,
Ain shams University.

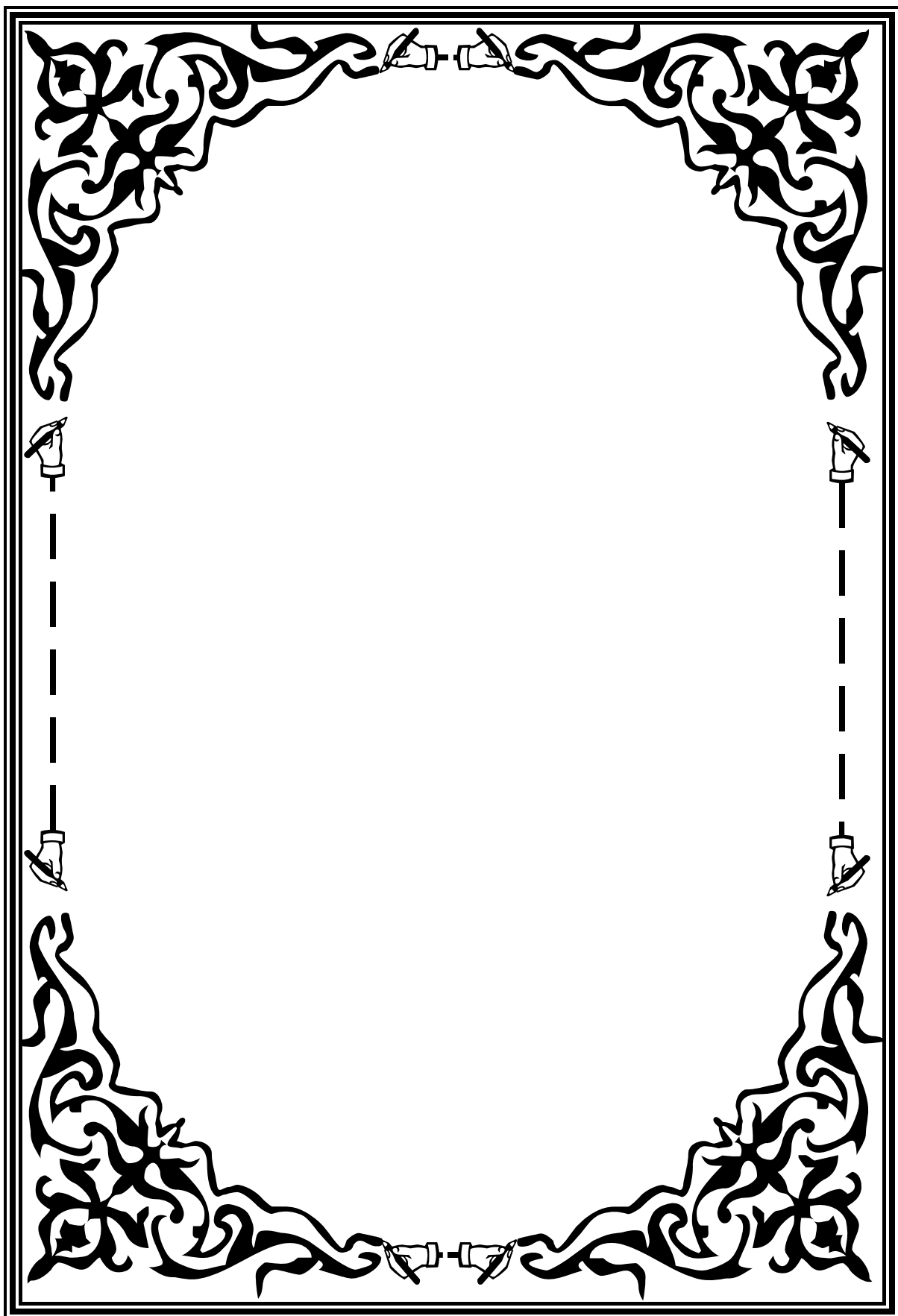
Dr. Abeer E. M. Zakaria

Lecturer of microbiology
National Center of Radiation
Research and Technology (NCRRT).

**Department of Microbiology
Faculty of science
Ain shams University
(2010)**

**This dissertation has not been previously
submitted for any degree at this or at any
other university**

Ali Mohamed Ali Saeed



ACKNOWLEDGEMENT

*First and foremost, I feel always indebted to
Allah, the most beneficent and merciful*

A great thank to Dr. Ahmed Osman Mostafa. Prof. of Biochemistry. Department of Biochemistry. Ain Shams University for his support, encouragement, valuable advices and constant help.

I would like to express my deep gratitude and thanks to my dear supervisor Dr. Khaled Zakaria El-Baghdady Lecturer of Microbiology, Department of Microbiology, Ain Shams University, for his help, encouragement, continuous advice, valuable suggestion in all steps of this thesis and his expert supervision. I am proud to be one of his students and I hope that he is satisfied with me.

I gratefully and sincerely thank my dear supervisor Dr. Abeer E. M. Zakaria Lecturer of Microbiology, National Center of Radiation Research and Technology (NCRRT) for her help, continuous support, valuable instructions and guidance from the start of the work. She was always patient, perfect in work organization and the best advisor.

I want to express my thanks to Dr. Mohamed Gad Abd El-Ghany Assistant Prof. of Microbiology, Department of Microbiology, Ain Shams University, for his continuous helps and encouragement.

A deep thank to Microbiology Department and all my Colleagues in Microbiology department for their assistance and support.

A very special thanks to My WIFE who has stood by me all through my studies and for her constant support and prayers.

Deep thanks to MY DAUGHTERS for their patience. Great thanks to My BROTHER for his support and continuous help from the start of my study.

And Finally, My deep appreciation goes to My ADORABLE PARENTS who learned me the first of everything in my life and without them, this work would not have seen light.

Eighteen oil degrading bacterial isolates were isolated from four different soil samples. Among them, 7 potent oil degrading bacterial isolates were selected on MSO medium (Mineral Silica-gel Oil medium).

Among the 7 selected isolates, 2 isolates LD10 and LD12 identified by 16S-rRNA genes as *Gordonia amicalis* and *Acinetobacter junii* respectively. These isolates showed maximum percentages of degradation on 3 used lubricant oils (Mobil, Mobil 1 and Caltex). However, consortia demonstrated better degradation than the isolates separately. Synthetic Mobil 1 was more degradable than Mobil and Caltex.

Several environmental conditions including incubation periods, pH, oil concentration, inoculum sizes, nitrogen & phosphorus concentrations and aeration were studied to enhance lubricant oils biodegradation by consortium (Clover cultivated field). The optimum environmental conditions obtained from this study showed better degradation for the used lubricant oils which was confirmed by FT-IR analysis. The degradation percentages reached 34.6 ± 2.3 , 45.2 ± 1.4 and 43.9 ± 0.7 for Mobil, Mobil 1 and Caltex respectively. FT-IR analysis of control and residual oil indicated the oxidation of oil which confirmed the modification of the original oil by microbial treatment.

CONTENTS

Subject	Page no.
List of tables	
List of figures	
Abbreviations	
Aim of work	
Abstract	1
Introduction	2
Literature Review	4
1-1 Biodegradation	5
1-2 Lubricant oils definition and structure	7
1-3 Lubricant oils pollution and toxicity	9
1-3-1 Sources of oil in the environment	9
1-3-2 Toxicity of used engine oil	10
1-4 Bacterial degradation of Lubricant oils	13
1-5 Bioremediation of oil polluted environments (Microbial enhanced oil remediation-MEOR)	16
1-5-1 Advantages of bioremediation	20
1-5-2 Disadvantages of bioremediation	21
1-6 Physical and chemical factors affecting the biodegradation of oil or hydrocarbons	22
1-6-1 Chemical composition of the oil	23

1-6-2 Physical state of the oil	24
1-6-3 Concentration of the Oil	27
1-6-4 Effect of Temperature	28
1-6-5 Effect of Oxygen level	29
1-6-6 Addition of Nutrients	31
1-6-7 Effect of pH	33
1-7 Biological factors affecting the biodegradation of oil or hydrocarbon	33
1-7-1 Adaptation effect of Prior Exposure	33
1-7-2 Microbial seeding	35
Materials and Methods	
2-1 Material	39
2-1-1 Base and Lubricant oils	39
2-1-2 Soil samples	39
2-1-3 Bacterial isolates	39
2-1-4 Culture media	40
2-1-5 Reagents and solutions	43
2-2 Methods	44
2-2-1 Soil samples	44
2-2-2 Lubricant oils samples	44
2-2-3 Isolation and enumeration of oil degrading bacteria	44
2-2-4 Preservation and maintenance of the pure bacterial isolates	45

2-2-5 Selection of the most promising oil degrading bacterial isolates	45
2-2-5-1 Examination of the bacterial isolates at different incubation periods and incubation temperatures	45
2-2-5-2 Examination of the bacterial growth on different base oil concentrations	46
2-2-6 Biodegradation of the used lubricant oils by selected isolates and consortia	46
2-2-7 Identification of the oil utilizing bacterial isolates by 16S-rDNA sequencing	47
2-2-8 Optimization of microbial degradation of lubricant oils	50
2-2-8-1 Incubation periods	50
2-2-8-2 pH	50
2-2-8-3 Concentrations of oil	51
2-2-8-4 Inoculum sizes	51
2-2-8-5 Nitrogen and phosphorus concentrations	52
2-2-8-6 Aeration conditions	53
2-2-9 Effect of biostimulation and bioaugmentation on microbial used lubricant oils degradation	54
2-2-10 Extraction of oil residues and gravimetric estimation	54
2-2-11 Fourier Transform Infrared (FT-IR) analysis	55
2-2-12 Statistical analysis	55

Results

3-1 Isolation and enumeration of oil degrading bacteria	56
3-1-1 Bacterial counts	56
3-1-2 Gross morphology	59
3-2 Selection of lubricant oil degrading bacteria isolates	62
3-2-1 Preliminary selection for oil degrading bacteria	62
3-2-2 Selection of the most potent oil degrading bacteria isolates	64
3-3 Biodegradation of the used lubricant oils by selected isolates and consortia	68
3-4 Microscopic examination of potent selected isolates	77
3-5 Identification of the oil utilizing bacterial isolates by 16S-rDNA sequencing	78
3-6 Optimization of microbial degradation of lubricant oils	81
3-6-1 Incubation period	81
3-6-2 pH	85
3-6-3 Oils concentrations	88
3-6-4 Inoculum sizes	91
3-6-5 Nitrogen and phosphorus concentrations	94
3-6-6 Aeration conditions	98
3-7 Effect of biostimulation and bioaugmentation on microbial used lubricant oils degradation	102
3-8 Growth rate of selected isolates and consortia on different lubricant oils	107

3-8-1 The growth rate of LD10 on different lubricant oils	107
3-8-2 The growth rate of LD12 on different lubricant oils	109
3-8-3 The growth rate of consortia on different lubricant oils	109
3-8-4 The growth rate of the combination between consortia and LD10 on different lubricant oils	110
3-8-5 The growth rate of the combination between consortia and LD12 on different lubricant oils	114
3-9 FT-IR analysis	116
Discussion	124
English summary	140
References	144
Arabic summary	

ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
BMM	Basal mineral medium
C	Caltex lubricant oil
CC	Caltex control
CFU	Colony Forming Units
EEC	European Economic Community
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
FT-IR	Fourier Transform Infrared analysis
g	Gram
LD	Lubricant Degrading bacteria
M	Mobil lubricant oil
M1	Mobil 1 lubricant oil
MC	Mobil control
M1C	Mobil 1 control
mg	milligram
ml	Milliliters
mM	Millimolar
µg	microgram
MSO	Mineral Silica-gel Oil medium
PAHs	Polycyclic aromatic hydrocarbons
PCR	Polymerase Chain Reaction