

**Investigation of the total petroleum hydrocarbon degrading microorganisms in
soil and water: a metagenomic approach**

Firouz Abbasian

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Philosophy**

**Global Centre for Environmental Remediation
Faculty of Science and Information Technology
The University of Newcastle**



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DECLARATION

I declare that:

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

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Signed_____

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TABLE OF CONTENTS

Abstract	1
Chapter 1: Introduction	2
Chapter 2. Literature review	4
2.1 Aliphatic Hydrocarbon Biodegradation by Bacteria	4
2.1.1. Introduction	4
2.1.2. Uptake of hydrocarbons into microbial cells	5
2.1.3. Metabolic shunts of n-Alkanes	6
2.1.4. Metabolism of alkenes	11
2.1.5. Branched chain alkanes	14
2.1.6 Cycloaliphatic compounds	15
2.1.7 Anaerobic hydrocarbon biodegradation	20
2.1.7.1 Denitrifying bacteria	21
2.1.7.2 Sulphate-reducing microorganisms	21
2.1.7.3 Metal oxidizing microorganisms	22
2.1.7.4 Intra-aerobic anaerobes	22
2.1.7.5 Methanogenesis	23
2.1.7.6 Anoxygenic sulphate dependent phototrophic bacteria	24
2.1.8. The Biochemistry of Anaerobic Hydrocarbon biodegradation	24
2.1.8.1 Fumarate Addition Reactions	24
2.1.8.2 Oxygen-Independent Hydroxylation	27
2.1.8.3 Carboxylation	28
2.1.8.4 Unsaturated bond (Alkene and Alkyne) Hydration	28
2.1.8.5 Reverse Methanogenesis	29
2.1.9. Application of hydrocarbon degrading enzymes in biotechnology	30
2.1.9.1 Chemicals and enzymes	30
2.1.9.2 Bioremediation	31
2.1.9.3 Biomarkers in oil biotechnology	32
2.1.10. Conclusion	33
2.2 The Genetics of Aliphatic and Aromatic Hydrocarbon Degradation	35
2.2.1 Introduction	35
2.2.2 The alkane degradation genes	36
2.2.3 Cycloaliphatic compounds	39

2.2.4 The plasmids containing naphthalene and salicylate degradation genes	40
2.2.5 The plasmids containing toluene and xylene oxidizing genes	45
2.2.6 Nitroaromatic compounds	50
2.2.7 Dibenzothiophene (DBT)	52
2.2.8 Anaerobic degradation of hydrocarbons	58
2.2.9 Anaerobic degradation of NACs	63
2.2.10 Genetics of microbial adaptation to high hydrocarbon concentrations	64
2.2.11 Conclusion	65
2.3 The Integration of Sequencing and Bioinformatics in Metagenomics	67
2.3.1 Introduction	67
2.3.2 Technical basis of metagenomics	68
2.3.3 Bioinformatics and Metagenomics	75
2.3.4 Applications of metagenomics in ecological studies	85
2.3.5 Applications of metagenomics in clinical studies	89
2.3.6 Application of metagenomic approach in biotechnology	92
2.3.7 Conclusion	95
Chapter 3. Experiments	98
3.1 A pyrosequencing-based analysis of microbial diversity governed by ecological conditions in the Winogradsky column	97
3.1.1 Abstract	97
3.1.2 Introduction	97
3.1.3 Material and method	99
3.1.3.1 Preparation of the Winogradsky column	99
3.1.3.2 DNA extraction and Pyrosequencing process	100
3.1.3.3 Analysis of data	101
3.1.3.4 Nucleotide sequence accession numbers	102
3.1.4 Results	102
3.1.4.1 Sequencing data	103
3.1.4.2 Alpha diversity	103
3.1.4.3 Classification of microorganisms based on their physiology	103
3.1.4.4 Classification of microorganisms based on their respiratory mechanism	105

3.1.5	Discussion	111
3.2	Effects of crude oil contamination on microbial diversity in the Winogradsky column as a fresh water lake biome	118
3.2.1	Abstract	118
3.2.2	Introduction	118
3.2.3	Material and methods	120
3.2.3.1	Preparation of the Winogradsky column and oil spiking	120
3.2.3.2.	TPH analysis in the crude oil contaminated sediments	120
3.2.3.3	DNA extraction and pyrosequencing process	121
3.2.3.4	Pyrosequencing data analysis	122
3.2.3.5	Nucleotide sequence accession numbers	122
3.2.4	Results and discussion	122
3.3	The biodiversity changes in the microbial population of soils contaminated with crude oil	136
3.3.1	Abstract	136
3.3.2	Introduction	136
3.3.3	Material and methods	137
3.3.3.1	Preparation of the column and oil spiking	137
3.3.3.2	DNA extraction and Pyrosequencing process	138
3.3.3.3	Data Analysis	138
3.3.3.4	Nucleotide sequence accession numbers	139
3.3.4	Results	139
3.3.5	Discussion	145
3.4	Multiwall carbon nanotubes increase the microbial community in crude oil contaminated fresh water sediments	149
3.4.1	Abstract	149
3.4.2	Introduction	149
3.4.3	Material and methods	151
3.4.3.1	Experimental setup	151
3.4.3.2	DNA extraction and pyrosequencing process	152
3.4.3.3	Data Analysis	152
3.4.3.4	Nucleotide sequence accession numbers	153

3.4.4	Results	153
3.4.5	Discussion	163
3.5	Microbial diversity and hydrocarbon degrading gene capacity of a crude oil field soil as determined by metagenomics analysis	168
3.5.1	Abstract	168
3.5.2	Introduction	168
3.5.3	Material and methods	170
3.5.3.1	TPH analysis	170
3.5.3.2	DNA sequencing using Illumina Hiseq platform and data analysis	171
	Nucleotide sequence accession numbers	172
3.5.4	Results	172
3.5.4.1	Gas chromatograph assays	172
3.5.4.2	Statistics of reads produced by HiSeq Illumina	172
3.5.4.3	The microbial communities in the crude oil well field	173
3.5.4.4	Classification of microorganisms based on their metabolism and respiration	173
3.5.5	Discussion	179
3.5.4.1	Alkane monooxygenases	182
3.5.4.2	Genes responsible for degradation of aromatic hydrocarbon	183
3.5.4.3	Genes responsible for desulfurization of sulfur-containing hydrocarbon	185
3.5.3.2.1	Genes responsible for microbial adaptation and growth in hydrocarbon contaminated soils	186
3.5.6	Conclusion	189
3.6	Microbial diversity and functional gene capacity of microorganisms in crude oil	190
3.6.1	Abstract	190
3.6.2	Introduction	190
3.6.3	Material and methods	191
3.6.3.1	TPH analysis	191
3.6.3.2	DNA extraction and DNA sequencing using the Illumina Hiseq platform	192

3.6.3.3 Data analysis	192
3.6.3.4 Nucleotide sequence accession numbers	193
3.6.5 Results and discussion	193
3.6.5.1 Gas chromatograph assays	193
3.6.5.2 Statistics of sequences produced by HiSeq Illumina	193
3.6.5.3 Phylogenetic compositions of the bacterial communities	194
3.6.5.4 Global gene expression and metabolic potential of the crude oil microbiome	198
3.6.5.5 Additional genes involved in microbial survival in crude oil	206
3.6.6 Conclusion	208
3.7 Identification of a new operon involved in desulfurization of Dibenzothiophenes using a metagenomic study and cloning and functional analysis of the genes	211
3.7.1 Abstract	211
3.7.2 Introduction	211
3.7.3 Material and Methods	212
3.7.3.1 Gene sequence and Primer design	212
3.7.3.2 Gene cloning and enzyme assay	213
3.7.3.3 Accession number	215
3.7.3.4 Results and discussion	215
Chapter 4. Summary, conclusions and outlooks	220
References	223

Abstract

Hydrocarbons are relatively recalcitrant compounds and are classified as high priority pollutants. However, these compounds are slowly degraded by a large variety of aerobic and anaerobic microorganisms. Although the corresponding genes in many phylogenetic groups of microbial species show different levels of diversity in terms of the gene sequence, the organisation of the genes in the genome or on plasmids and the activation mode of several microorganisms show identical hydrocarbon degrading genes.

Since the majority of microorganisms in natural environments cannot be cultured in laboratory media, culture-based systems are unable to estimate the full microbial diversity of an environment. Metagenomic methods, however, employ sequencing procedures for the determination of the microbial diversity of a community and for examining a particular functional ability of microorganisms in the environment using genomic DNA obtained directly from environmental samples. Application of metagenomic methods provides a huge amount of data that can be analysed only by using powerful computational bioinformatics tools.

In this study, we used next generation technology and metagenomic analysis to investigate the microbial diversity in crude oil and crude oil contaminated soils and to find the functional genes involved in the degradation of hydrocarbons in crude oil. The findings from this study can be used for bioremediation of crude oil spills and also for improvement of the quality of crude oil derivatives in terms of removal of sulfur and nitrogen. As a part of this study, we report a list of microorganisms that are abundant in the crude oil and the crude oil contaminated soil. Furthermore, we found a new operon responsible for removal of sulfur from dibenzothiophenes. The three genes in this operon were cloned and their activities measured in cell free condition.