CONTENTS

Chapter	Description	Page no
Declaration		
Certificate		
Abstract		
Preface		
List of tables		
List of figures		
Appendices		
a. List of abbreviation		
b. Thesis related publications		
1.	**General introduction and review of literature**	
1.1	**Introduction**	1
1.2	**Objectives**	3
1.3	**Review of the literature**	
	1.3.1. Structure and classification of petroleum	4
	1.3.2. Recent scenario of petroleum spills and its hazardous effect	5
	1.3.3. Remediation processes	7
	1.3.4. Factors involved in petroleum hydrocarbon degradation	10
	1.3.5.Microorganisms involved in petroleum hydrocarbon degradation	16
	1.3.6. Metabolism of petroleum hydrocarbon	20
	1.3.7. Microbial physiological response to petroleum hydrocarbon	29
	1.3.8. Recent advances in petroleum degradation	31
	1.3.9.Application of petroleum degrading microorganism for biodiesel production	37
1.4	**Bibliography**	39
2.	**Degradation of petroleum hydrocarbon by isolated bacterial cells**	
2.1	**Introduction**	48
2.2	**Materials and Methods**	
	2.2.1. Isolation of petroleum hydrocarbon degrading microorganisms from soil sample	50
	2.2.2. Screening of the best petroleum hydrocarbon degrading microbial strain	50
	2.2.3.Phenotypic and Biochemical characterization of the selected bacterial strain	50
	2.2.4.Antibiogram of the selected bacterial strain	51

2.2.5. Phylogenetic characterization of the selected bacterial strain
2.2.5.1. Isolation of genomic DNA 51
2.2.5.2. Agarose gel electrophoresis of isolated DNA 51
2.2.5.3. PCR amplification of 16S rRNA and its sequence analysis 52
2.2.6. Determination of growth and dry cell mass of PS11strain 52
2.2.7. Tolerance of PS11 cells to other solvents 52
2.2.8. Transmission electron microscopy (TEM) of PS11 in presence of petroleum hydrocarbon: 53
2.2.9. Determination of membrane fatty acid adaptive profile in presence of petroleum hydrocarbons 54
2.2.9.1. Growth conditions
2.2.9.2.Extraction of bacterial membrane lipid
2.2.9.3. Phospholipid analysis
2.2.9.4. Analysis of fatty acid composition by gas chromatography
2.2.10. Detection of total petroleum hydrocarbons (TPH) and some individual petroleum hydrocarbon degradation by Gas Chromatography (GC) 55
2.2.11. Characterization of the gene responsible for petroleum hydrocarbon degradation.
2.2.11.1. Isolation of plasmid DNA and its Restriction digestion 55
2.2.11.2. Curing of plasmid DNA 56
2.2.11.3. Growth of plasmid cured and wild PS11 strain in presence of petroleum hydrocarbon 56
2.2.11.4. Preparation of competent E. coli JM109 57
2.2.11.5. Transformation of plasmid in JM109 and screening of transformed cells 57
2.2.11.6. Degradation of Total petroleum hydrocarbons (TPH) and some individual petroleum hydrocarbon by transformed JM109 cells 58
2.2.11.7. PCR amplification and sequencing of plasmid borne gene responsible for degradation 58

2.3 Results

2.3.1. Isolation of petroleum hydrocarbon degrading microbial strain 60
2.3.2. Screening of best petroleum hydrocarbon degrading microbial strain 60
2.3.3 Biochemical and phylogenetic characterization of the selected strain 62
2.3.4 Determination of growth and dry cell mass of *G. stearothermophilus* PS11 65
2.3.5 Tolerance property of *G. stearothermophilus* PS11 to other solvents 66

2.3.6 Transmission electron microscopy(TEM) analysis of *G. stearothermophilus* PS11 in presence of petrol 67

2.3.7 Determination of membrane fatty acid adaptive profile in presence of petroleum hydrocarbons

2.3.7.1. Phospholipid analysis 68

2.3.7.2. Analysis of fatty acid composition 69

2.3.8. Detection of Total petroleum hydrocarbons (TPH) and some individual petroleum hydrocarbon degradation by gaschromatography (GC) 71

2.3.9. Characterization of the gene responsible for petroleum hydrocarbon degradation.

2.3.9.1. Isolation of plasmid DNA and restriction digestion profile 73

2.3.9.2. Curing of plasmid DNA 73

2.3.9.3. Growth of plasmid cured strain and wild type strain in presence of petroleum hydrocarbon 74

2.3.9.4. Transformation of plasmid in JM109 and screening of transformed cell 75

2.3.9.5. Degradation of Total petroleum hydrocarbons (TPH) and some individual petroleum hydrocarbon by transformed JM109 cells 76

2.3.9.6. PCR amplification and sequencing of plasmid borne gene responsible for degradation 77

2.3.9.7. Sequence comparison of C23O with other extradiol dioxygenases 79

2.4 Discussion 80

2.5 Bibliography 88

3. Application of PS11 cells for biodiesel production

3.1 Introduction 92

3.2 Materials and Methods

3.2.1 Determination of lipase production by PS 11 94

3.2.2 Production media, enzyme preparation and measurement of bacterial growth 94

3.2.3 Lipase assay 94

3.2.4 Optimization of PS11 lipase production by OVAT method 95

3.2.5 Purification of PS11 lipase and determination of 95

molecular weight
3.2.6 Characterization of purified PS11 lipase

3.2.6.1. Effect of pH on activity and stability of PS11 lipase 96
3.2.6.2. Effect of temperature on activity and stability of PS11 lipase 96
3.2.6.3. Effect of metal ions and chemicals on PS 11 lipase activity 96
3.2.6.4. Effect of detergents on lipase stability 96
3.2.6.5 Effect of organic solvent on lipase stability 97

3.2.7 Application of lipase PS11 in biodiesel production
3.2.7.1. Sample preparation 97
3.2.7.2. Determination of biodiesel production by TLC method 97
3.2.7.3.Determination of biodiesel production by gas chromatography 98

3.3 Results

3.3.1 Kinetics of lipase production by *Geobacillus stearothermophilus* PS11 99
3.3.2 Optimization of PS11 lipase production by OVAT method 99
3.3.3 Purification of PS11 lipase and determination of molecular weight 100
3.3.4 Effect of pH and temperature on activity and stability of PS11 lipase 102
3.3.5 Effect of metal ions and chemical reagents on PS 11 lipase activity 103
3.3.6 Effect of detergents on lipase stability \ 103
3.3.7 Effect of solvents on lipase stability 104
3.3.8 Application of lipase PS11 in biodiesel production
3.3.8.1. Determination of biodiesel production by TLC method 105
3.3.8.2.Determination of biodiesel production by GC method 106

3.4 Discussion 108
3.5 Bibliography 114
3.6 Conclusion 118