



UNIVERSITI PUTRA MALAYSIA

**BIODEGRADATION OF PETROLEUM SLUDGE BY
*METHYLOBACTERIUM SP.***

ZAKUAN AZIZI BIN SHAMSUL HARUMAIN

FBSB 2012 4

**BIODEGRADATION OF PETROLEUM SLUDGE BY
*METHYLOBACTERIUM SP.***

BY

ZAKUAN AZIZI BIN SHAMSUL HARUMAIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
in Fulfilment of the Requirement for the Degree of Master of Science**

September 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

**BIODEGRADATION OF PETROLEUM SLUDGE BY
*METHYLOBACTERIUM SP.***

By

ZAKUAN AZIZI BIN SHAMSUL HARUMAIN

Chairman : Associate Professor Dr. Mohd Yunus Abd Shukor, PhD

Faculty : Biotechnology and Biomolecular Sciences

Abstract: A bacterium was isolated from sludge contaminated soil in petroleum refinery centre and tested for its ability to degrade hydrocarbon in petroleum sludge obtained from Shell Refinery Centre, Port Dickson, Negeri Sembilan. The isolate was tentatively identified as *Methylobacterium* sp. strain ZASH based on the partial 16s rRNA molecular phylogeny. The optimum condition for the strain to degrade petroleum sludge was characterized and quantified using GCFID. It was revealed that the bacterium degrade optimally between the temperature of 30°C to 35°C, pH 7 to 7.5, 0.5 to 1.5% (v/v) Tween 80 as surfactant source and at 1 to 2% (w/v) peptone as the nitrogen source. Gas chromatography analysis revealed that after 15 days, the *Methylobacterium* sp. strain ZASH was able to degrade 70% of hydrocarbons component of chain length C12-C36 found in petroleum sludge. It was also found that sawdust can be a good hydrocarbon adsorbent as its addition increase the hydrocarbon removal up to 99% removal.

Keywords: Petroleum Sludge, Sawdust, *Methylobacterium* sp.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**BIOPENGURAIAN ENAPCEMAR PETROLEUM OLEH
*METHYLOBACTERIUM SP.***

Oleh

ZAKUAN AZIZI BIN SHAMSUL HARUMAIN

Pengerusi : Profesor Madya Dr. Mohd Yunus Abd Shukor, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Abstrak: Sejenis bakteria telah dipencilkan daripada bahan enapcemar petroleum yang diperoleh daripada pusat penapisan petroleum dan telah diuji untuk melihat kebolehan bakteria tersebut menguraikan sebatian hidrokarbon dalam bahan enapcemar petroleum yang diperoleh dari Pusat Penapisan Minyak Shell, Port Dickson, Negeri Sembilan. Bacteria itu dikenalpasti sebagai *Methylobacterium sp.* strain ZASH berdasarkan kajian filogeni terhadap sebahagian molekul 16s rRNA. Kondisi optimum bagi penguraian sebatian enapcemar petroleum dikaji dan dianalisis menggunakan GCFID. Kajian mendedahkan bakteria tersebut hidup dan menguraikan sebatian hidrokarbon secara optimum diantara suhu 30°C hingga 35°C, pH 7 hingga 7.5, kepekatan tween 80 sebagai sumber surfaktan pada 0.5 kepada 1.5% (v/v) dan kepekatan pepton sebagai sumber nitrogen pada 1 hingga 2% (w/v). Analisis kromatografi mendedahkan bahawa bakteria ini berupaya menguraikan sebatian hidrokarbon bersaiz C12 hingga C36 sebanyak 70% penyingkiran. Kajian juga mendapati bahawa habuk papan sesuai untuk menjadi bahan penyerap sebatian hidrokarbon dimana penambahan habuk papan telah meningkatkan penyingkiran hidrokarbon sebanyak 99% penyingkiran.

Katakunci: Enapcemar Petroleum, Habuk Papan, *Methylobacterium sp.*

ACKNOWLEDGMENT

In the name of Allah, the most merciful and the most compassionate. First and foremost, the author would like to thank Allah for giving him the opportunity to complete this research and for guiding him throughout his life up until now. Secondly, the author would like to express his deepest gratitude towards his supervisor, Associate Professor Dr. Mohd Yunus Abdul Shukor, as well as his co supervisor, Professor Dr. Mohd Arif Syed for helping and guiding him throughout the research period. The author would also like to thank to all the staff of Faculty of Biotechnology UPM and to his fellow lab members, Ezuan, Badrin, Maryam, Gan and others, for providing him with such a great working environment, assistance and also for the knowledge that has been shared. Not to forget, the author would like to show his appreciation towards Brother Noor Faizul Hadry for his extraordinary ideas, assistance and thoughts. He would also like to thank to the Ministry of Higher Education for their financial support. Special thanks to his parents, parents in law, brothers, sisters and to all his family members for their continuous motivation and prayers. Last but not least, the author would like to say thanks to his lovely wife, Madam Ilham Hazizan for being such a great wife, a dedicated mother, a true listener, a good motivator and truly understanding. May this masterpiece be the stepping stone of his success in his career as a scientist.

*Specially dedicated to my dedicated parents, my lovely wife, my beautiful family
and to my beloved daughter,*

'Ilma binti Zakuan Azizi

Rabbi Zidni 'Ilma

"My Lord! Increase me in knowledge."

I certify that an Examination Committee has met on 13 September 2012 to conduct the final examination of Zakuan Azizi Bin Shamsul Harumain on his Master of Science thesis entitled “Biodegradation of petroleum sludge by *Methylobacterium* sp. strain ZASH” in accordance with Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Mohd Yunus Abdul Shukor, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Mohd Arif Syed, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

BUJANG KIM HUAT, PhD

Professor/ Dean

School of Graduate Studies

Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.

ZAKUAN AZIZI BIN SHAMSUL HARUMAIN

Date:



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENTS	iv
APPROVAL	vii
DECLARATION	viii
TABLE OF CONTENTS	ix
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xviii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 Petroleum Sludge	
2.1.1 Physical and Chemical Properties of Petroleum Sludge	4
2.1.1.1 Asphaltenes	6
2.1.1.2 Aromatics	7
2.1.1.3 Alkanes	8
2.1.2 Sources of Petroleum Sludge	9
2.2 Toxicity of Petroleum Sludge	
2.2.1 Effects on the Environment and Other Organisms	10
2.3 Bioremediation	
2.3.1 Bioremediation Technology	11
2.3.2 Advantages of Bioremediation	12
2.4 Petroleum Sludge Treatment	
2.4.1 Current techniques on Petroleum Sludge Management	13
2.4.2 Microorganisms and Petroleum Sludge Biodegradation	16
2.4.2.1 Petroleum Sludge degrading bacteria	17
2.4.2.2 Petroleum Sludge degrading fungi	18
2.4.3 Culture condition in microbial degradation of Petroleum Sludge	19

	2.4.3.1 Effect of hydrocarbon availability on biodegradation of Petroleum Sludge	19
	2.4.3.2 Effect of pH on biodegradation of Petroleum Sludge	20
	2.4.3.3 Effect of temperature on biodegradation of Petroleum Sludge	20
	2.4.3.4 Effect of nitrogen sources on Biodegradation of Petroleum Sludge	21
	2.4.3.5 Effect of surfactant on biodegradation of Petroleum Sludge	22
	2.4.4 Mechanism of biodegradation of petroleum sludge by bacteria	25
	2.4.4.1 Mechanism of biodegradation of aliphatic hydrocarbon	29
	2.5 The Genus <i>Methylobacterium</i>	31
	2.6 Effect of sawdust as bulking agent	33
3	MATERIALS AND METHODS	
	3.1 Chemicals	36
	3.1.1 Culture Medium	36
	3.1.2 Isolation and screening of petroleum sludge-degrading bacteria	37
	3.1.3 Petroleum sludge analysis	38
	3.1.4 Total petroleum hydrocarbon fractionation	38
	3.1.4.1 Gas chromatography flame ionization detector analysis	39
	3.1.5 Determination of petroleum sludge degrading bacterial growth profile	40
	3.2 Identification of petroleum sludge degrading bacterium	40
	3.2.1 Gram Staining	41
	3.2.2 Oxidase Test	42
	3.2.3 Catalase Test	42
	3.2.4 16S rRNA analysis	43
	3.2.4.1 Genomic Extraction	43
	3.2.4.2 Polymerase Chain Reaction (PCR)	44
	3.2.4.3 Purification of Amplified PCR Product	45
	3.2.4.4 Sequence Analysis	45
	3.2.4.5 Phylogenetic Analysis	46
	3.2.4.6 Maintenance of bacterial isolate	47
	3.3 Characterization of <i>Methylobacterium</i> sp. strain ZASH	47
	3.3.1 Effect of temperature on bacterial growth and petroleum sludge degradation	48
	3.3.2 Effect of pH on bacterial growth and petroleum sludge degradation	48
	3.3.3 Effect of nitrogen sources on bacterial growth and petroleum sludge degradation	49
	3.3.4 Effect of nitrogen concentration on bacterial growth and petroleum sludge degradation	50
	3.3.5 Effect of surfactant sources on bacterial growth and petroleum sludge degradation	51

	3.3.6 Effect of surfactant concentration on bacterial growth and petroleum sludge degradation	52
	3.3.7 Effect of carbon concentration on bacterial growth and petroleum sludge degradation	53
	3.3.8 Growth and degradation kinetics	54
	3.3.8.1 Biodegradation kinetics model	54
3.4	Biodegradation profile of petroleum sludge by <i>Methylobacterium</i> sp. strain ZASH	55
3.5	Effect of sawdust on biodegradation of hydrocarbon in petroleum sludge	56
	3.5.1 Characterization of sawdust	56
	3.5.2 Effect of sawdust concentration on bacterial growth and petroleum sludge degradation	57
	3.5.3 The effect of sawdust on adsorption of hydrocarbons and biodegradation of hydrocarbons in petroleum sludge by <i>Methylobacterium</i> sp. strain ZASH.	58
3.6	Statistical analysis	58
4	RESULTS AND DISCUSSIONS	
4.1	Isolation and Screening of Petroleum Sludge-degrading bacteria	59
4.2	Petroleum sludge analysis	61
	4.2.1 Total petroleum hydrocarbon fractionation	61
4.3	Determination of petroleum sludge degrading bacterial growth profile	63
4.4	Identification of isolate ZASH	65
	4.4.1 Gram staining	65
	4.4.2 Catalase test	65
	4.4.3 Oxidase test	65
	4.4.4 16s rDNA Analysis	67
	4.4.4.1 Genomic Extraction	67
	4.4.4.2 Polymerase Chain Reaction (PCR)	67
	4.4.4.3 16s rDNA Gene Sequencing	67
	4.4.4.4 Phylogenetic Analysis	70
4.5	Characterization of bacterial growth and petroleum sludge degradation	
	4.5.1 The effect of temperature on bacterial growth and petroleum hydrocarbon degradation	72
	4.5.2 The effect of pH on bacterial growth and petroleum hydrocarbon degradation	75
	4.5.3 The effect of nitrogen source on bacterial growth and petroleum hydrocarbon degradation	78
	4.5.4 The effect of peptone concentration on bacterial growth and petroleum hydrocarbon degradation	81
	4.5.5 The effect of surfactant source on bacterial growth and petroleum hydrocarbon degradation	84
	4.5.6 The effect of tween 80 concentration on bacterial growth and petroleum hydrocarbon degradation	87
	4.5.7 The effect of petroleum sludge concentration on bacterial growth and petroleum hydrocarbon degradation	90

4.5.8 Biodegradation profile of hydrocarbon in petroleum sludge by <i>Methylobacterium</i> sp. strain ZASH	97
4.5.9 Growth and degradation kinetics	102
4.6 The effect of sawdust addition on bacterial growth and biodegradation of petroleum sludge	102
4.6.1 The effect of sawdust concentration on bacterial growth and biodegradation of petroleum sludge	104
4.6.2 The effect of sawdust on removal and biodegradation of petroleum sludge	110
5 CONCLUSION	112
REFERENCES	129
APPENDICES	135
BIODATA OF THE AUTHOR	

