

UNIVERSITI TEKNOLOGI MARA

**POLYCYCLIC AROMATIC
HYDROCARBONS
BIODEGRADATION USING
ISOLATED STRAINS UNDER
INDIGENOUS CONDITION**

NORZILA OTHMAN

Thesis submitted in fulfillment of the requirements

for the degree of

Doctor of Philosophy

Faculty of Civil Engineering

November 2010

CANDIDATE'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

In the event my thesis be found to violate the conditions mentioned above, I voluntarily waive the right of conferment of my degree and agree be subjected to the disciplinary rules and regulations of Universiti Teknologi MARA.

Name of Candidate	NORZILA BINTI OTHMAN
Candidate's ID No.	2005102959
Programme	DOCTOR OF PHILOSOPHY IN CIVIL ENGINEERING (EC 990)
Faculty	FACULTY OF CIVIL ENGINEERING
Thesis Title	POLYCYCLIC AROMATIC HYDROCARBONS BIODEGRADATION USING ISOLATED STRAINS UNDER INDIGENOUS CONDITION
Signature of Candidate	 _____
Date	NOVEMBER 2010

ABSTRACT

The treatment and disposal of domestic sludge is an expensive and environmentally sensitive problem. It is also a growing problem since sludge production will continue to increase as new wastewater treatment plants are built due to population increase. The large volume of domestic sludge produced had made it difficult for many countries including Malaysia to assure complete treatment of the sludge before discharging to the receiving environment. Domestic sludge contains diverse range of pollutants such as pathogen, inorganic and organic compounds. These pollutants are toxic, mutagenic or carcinogenic and may threaten human health. Improper disposal and handling of sludge may pose serious impact to the environment especially on soil and water cycles. Previous studies on Malaysian domestic sludge only reported on bulk parameters and heavy metals. Thus, no study reported on organic micro pollutants, namely, polycyclic aromatic hydrocarbons (PAHs). Their recalcitrance and persistence make them problematic environmental contaminants. Microbial degradation is considered to be the primary mechanism of PAHs removal from the environment. Much has been reported on biodegradation of PAHs in several countries but there is a lack of information quantitative on this subject in Malaysia. This study is carried out to understand the nature of domestic sludge and to provide a better understanding on the biodegradation processes of PAHs. The methodology of this study comprised field activities, laboratory work and mathematical modelling. Field activities involved sampling of domestic sludge from Kolej Mawar, Universiti Teknologi MARA, Shah Alam, Selangor. Laboratory activities include seven phases of experimental works. First phase is characterization study of domestic sludge based on bulk parameters, heavy metals and PAHs. Second phase is enrichment and purification of bacteria isolated from domestic sludge using single PAHs and mixed PAHs as growth substrate. This was followed by identification of bacteria using BIOLOG system. The fourth phase focussed on turbidity test to monitor growth rate of the isolated bacteria. Preliminary degradation study involves optimization of the process at different substrate concentration, bacteria concentration, pH and temperature. The optimum conditions established from optimization study were used in degradation study. In biodegradation study, two experimental conditions were performed. These conditions include using bacteria isolated from single PAHs as substrate and bacteria isolated from mixed PAHs. Protein and pH tests were done during degradation study. Final activity is mathematical modelling of the biodegradation process. In general results on bulk parameters are comparable to previous studies. Zinc was the main compound with a mean concentration of 1196.4 mg/kg. PAHs were also detected in all of the samples, with total concentration between 0.72 to 5.36 mg/kg dry weight for six PAHs. In the examined samples, phenanthrene was the main compound with a mean concentration of 1.0567 mg/kg. The results from purification studies of bacteria strains successful isolated 13 bacteria strains from single PAH substrate while three bacteria were isolated from the mixed PAHs substrate. Based on bacteria growth rates, only six strains grown on single PAHs and three strains grown on mixed PAHs were used for further studies. Results from the optimization study of biodegradation indicated that maximum rate of PAHs removal occurred at 100 mgL⁻¹ of PAHs, 10% bacteria concentration, pH 7.0 and 30°C. The results showed that bacteria grown on lower ring of PAHs are not able to grow on higher ring of PAHs. As for example *Micrococcus diversus* grown on naphthalene as sole carbon source was unable to degrade other PAHs like acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene. In the case of bacteria isolated from mixed PAHs, the results showed that most of the naphthalene was degraded by isolated strains with the highest average degradation rate followed by acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene.

Different degradation trends were observed in the study could be attributed to the different substrate provided during isolation process. Interaction through cometabolism and synergistic occurred for bacteria strains isolated from single substrate. Thus, only synergistic interaction was observed for bacteria isolated from mixed substrate. *Corynebacterium urolyticum* revealed to be the best strain in degrading PAHs. The experimental results have led to a model concept describing PAHs degradation.

ACKNOWLEDGEMENTS

I am extremely grateful to my supervisor, Prof. Sr. Ir. Dr. Hj. Suhaimi Abdul Talib and Assoc. Prof. Dr. Noor hana Hussain for their support, encouragement, inspiration and guidance at all stages of my PhD program at UiTM.

I also owe my gratitude to University Tun Hussein Onn Malaysia and University Technology Mara for the financial and technical support.

I would like to express my sincere gratitude to my friends Fiza, Amani, Tay, Liew, Saliha, Sheela for generously helping me with any problem I ran across along my PhD journey. I thank all the technicians for their help during laboratory works. I would like to thank all my friends at UTHM and UiTM for their support.

Above all, to my parents for the emotional support and blessing. To my lovely husband Irwan and my sons, Irfan and Irsyad who have been an inspiration to me. Without the blessing of them, I would not have made it through.