



UNIVERSITI PUTRA MALAYSIA

**BIODEGRADATION OF DIESEL BY LOCAL ISOLATE
BACILLUS PUMILUS STRAIN NHK**

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BACILLUS PUMILUS STRAIN NHK**



By

NURULHUDA BINTI KAIDA

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

June 2012



Dedicated to my family...

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

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Chairman: Prof. Mohd Arif Bin Syed, PhD

Faculty : Biotechnology and Biomolecular Sciences

Petroleum hydrocarbon such as diesel has become one of the potential sources of environmental contamination in Malaysia. The abundance of diesel in coastal areas and soils cause massive pollution and must be eliminated due to their high toxicity effects. Therefore, this study is about the use of microorganism to alleviate diesel pollution as an alternative technology of chemical- or physical-based oil-pollution treatments. Biodegradation of diesel requires a diesel-tolerant microbe to reduce degradation time. Local bacteria isolated from oil-contaminated soil and water from various locations in Malaysia were screened for their ability to degrade large amount of diesel in a shorter time. Enrichment culture from soil samples gave several isolates capable of degrading diesel. By using gravimetric method, Isolate T5 was selected for further studies because it has been shown to have high diesel-degradation ability in a shorter period and able to use diesel as its carbon and energy source in contrast to the other isolates. The isolate was identified as *Bacillus pumilus* Strain NHK (1439 bp) (99% sequence homology) by using 16S rRNA molecular phylogenetic analysis. In this study, the isolate exhibited

optimum diesel degradation and growth in media containing 5% (v/v) diesel concentration at 30 °C, 0.9% (w/v) of sodium nitrate (NaNO₃) as an additional nitrogen source, optimum pH 7.5 (phosphate buffer) and in the presence of 0.01% (v/v) Tween 80 as a surfactant. Gravimetric method revealed that Isolate T5 has been proven to show high diesel-degradation ability and almost 75% of diesel was degraded by Isolate T5 in 20 days as further proven when analyzed using gas chromatography-flame ionization detector (GC-FID). After just 4 days of diesel degradation studies, the biodegradation efficiency rate has significantly increased from 26.7 to 37% with a total increment of 10.3% after growth optimization. The effects of various heavy metal and salinity on diesel degradation were tested in this experiment. The presence of zinc at 5 ppm enhanced diesel degradation by about 18% as compared to control sample while the other heavy metal caused minor inhibition on the diesel degradation and growth of Isolate T5 after 4 days. The best concentration of sodium chloride (NaCl) for diesel degradation was 0 to 4% (w/v). Isolate T5 was then immobilized on gellan gum with the optimum immobilization parameters of 0.75% (w/v), bead's size of 3 mm diameter and bead number of 30 per 10 ml medium. Diesel degradation was compared between free cells and gellan gum immobilized cells. From the results obtained, at diesel concentration of 5% (v/v), gellan gum immobilized cells degraded diesel oil faster with 96% degradation compared to 83.6% degradation for free cells in 10 days of incubation. The bacterium's characteristics meet the requirements for bioremediation of diesel accidents and pollutions either in soil or coastal area co-contaminated with heavy metals in the tropics. The outcome of this study will contribute towards the knowledge in diesel degradation process in order to support environmental sustainability as well as

minimizing costs if conventional technology was used especially for a developing country like Malaysia.



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

**BIODEGRADASI DIESEL OLEH ISOLAT TEMPATAN *BACILLUS PUMILUS*
STRAIN NHK**

Oleh

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Hidrokarbon petroleum seperti diesel telah menjadi salah satu sumber yang berpotensi terhadap pencemaran alam sekitar di Malaysia. Diesel yang banyak di kawasan pantai dan tanah menyebabkan pencemaran yang teruk dan mesti dihapuskan kerana kesan ketoksikan diesel yang tinggi. Oleh itu, kajian ini meliputi penggunaan mikroorganisma untuk mengurangkan pencemaran hidrokarbon sebagai teknologi alternatif kepada rawatan pencemaran minyak berasaskan kimia atau fizikal. Biodegradasi diesel memerlukan mikrob yang toleransi terhadap diesel untuk mengurangkan masa degradasi. Bakteria tempatan yang dipencilkan daripada tanah dan air yang tercemar yang diambil dari beberapa lokasi di Malaysia telah disaring terhadap keupayaan mikroorganisma tersebut untuk mendegradasi jumlah diesel yang banyak dalam masa yang singkat. Isolat yang berkebolehan untuk mendegradasi diesel telah diperolehi daripada teknik peningkatan pertumbuhan kultur. Dengan menggunakan kaedah gravimetrik, Isolat T5 telah dipilih untuk kajian lanjutan kerana telah terbukti mempunyai keupayaan mendegradasi diesel yang tinggi dalam tempoh yang singkat dan boleh menggunakan

hidrokarbon sebagai karbon dan sumber tenaga berbanding isolat yang lain. Melalui penganalisaan filogenetik molekul 16S rRNA, isolat ini telah dikenalpasti sebagai *Bacillus pumilus* Strain NHK (1439 bp) (99% homologi jujukan). Dalam kajian ini, isolat ini telah mempamerkan kadar degradasi diesel dan pertumbuhan yang optimum di dalam media yang mengandungi kepekatan diesel sebanyak 5% (v/v) pada suhu 30 °C, 0.9% (w/v) natrium nitrat (NaNO₃) sebagai sumber nitrogen tambahan, pH optimum pada 7.5 (penimbal fosfat) dan dalam kehadiran 0.01% (v/v) Tween 80 sebagai surfaktan. Kaedah gravimetrik telah menunjukkan bahawa Isolat T5 telah terbukti mempunyai keupayaan mendegradasi diesel yang tinggi dengan hampir sebanyak 75% diesel telah didegradasi dalam tempoh 20 hari dan telah dibuktikan dengan lebih lanjut apabila dianalisis dengan menggunakan ‘gas chromatography-flame ionization detector’ (GC-FID). Selepas hanya 4 hari kajian degradasi diesel, didapati kadar kecekapan biodegradasi telah meningkat dengan ketara sebanyak 26.7 kepada 37% dengan jumlah kenaikan sebanyak 10.3% selepas pengoptimuman pertumbuhan isolat. Kesan terhadap pelbagai logam dan kemasinan terhadap degradasi diesel juga telah diuji dalam eksperimen ini. Kehadiran zink pada kepekatan 5 ppm meningkatkan degradasi diesel kira-kira 18% berbanding sampel kawalan manakala kehadiran logam lain menyebabkan perencatan yang kecil terhadap degradasi diesel dan pertumbuhan Isolat T5 selepas 4 hari. Kepekatan natrium klorida (NaCl) iaitu pada 0 hingga 4% (w/v) adalah kepekatan terbaik untuk degradasi diesel. Isolat T5 kemudian telah disekatgerakkan dengan menggunakan gellan gum dan mempunyai parameter optimum pada 0.75% (w/v), saiz manik berdiameter 3 mm dan sebanyak 30 biji manik dalam media berukuran 10 ml. Degradasi diesel telah dibandingkan di antara sel-sel bebas dan sel-sel yang telah disekatgerak dengan gellan gum. Daripada keputusan yang diperolehi, pada kepekatan

diesel sebanyak 5% (v/v), sel- sel yang telah disekatgerak dengan gellan gum lebih cepat mendegradasi diesel sebanyak 96% berbanding sel-sel bebas sebanyak 83.6% dalam tempoh sepuluh hari. Ciri-ciri yang ada pada bakteria ini memenuhi keperluan untuk bioremediasi diesel akibat kemalangan dan pencemaran di sekitar tanah atau kawasan pantai yang tercemar bersama logam di kawasan tropika. Hasil kajian ini akan menyumbang ke arah pengetahuan dalam proses pendegradasian diesel untuk menyokong kemampuan alam sekitar serta mengurangkan kos sekiranya teknologi konvensional digunakan terutama bagi sebuah negara membangun seperti Malaysia.



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I certify that a Thesis Examination Committee has met on 27 June 2012 to conduct the final examination of Nurulhuda Bt Kaida on her Master of Science thesis entitled “Biodegradation of Diesel by Local Isolate *Bacillus Pumilus* Strain NHK” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.A (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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Date: 27 June 2012

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