APPLICATION OF MYCOREMEDIATION IN SPENT ENGINE OIL

HO CHENG CHENG

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA

2017

NON-PLAGIARISM DECLARATION

By this letter I declare that I have written this thesis completely by myself, and that I have used no other sources or resources than the ones mentioned.

I have indicated all quotes and citations that were literally taken from publications, or that were in close accordance with the meaning of those publications, as such. All sources and other resources used are stated in the references.

Moreover I have not handed in a thesis similar in contents elsewhere.

In case of proof that the thesis has not been constructed in accordance with this declaration, the Faculty of Health and Life Sciences has the right to consider the research thesis as a deliberate act that has been aimed at making correct judgment of the candidate's expertise, insights and skills impossible.

I acknowledge that the assessor of this item may, for the purpose of assessing this item,

- reproduce this assessment item and provide a copy to another member of the University; and/or,
- communicate a copy of this assessment item to a plagiarism checking service (which may then retain a copy of the assessment item on its database for the purpose of future plagiarism checking).

In case of plagiarism the examiner has the right to fail me and take action as prescribed by the rules regarding Academic Misconduct practiced by INTI International University.

Ho Cheng Cheng	Cc
Name	Signature
I15007651	14 JULY 2017
I.D.Number	Date

DECLARATION

I hereby declare that the work in this dissertation is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Dr. Ong Ghim Hock.

Ho Cheng Cheng	*Dr. Ong Ghim Hock*
I15007651	(SUPERVISOR)
14 JULY 2017	

ACKNOWLEGDEMENT

It had been a challenging but fun journey working on this thesis. First, I would like to express sincere gratitude to my family for being there for me all the time, supporting me morally and financially along the way. Next, I would like to thank all the lecturers and lab assistants for their help in completing this research project. Without their assistance, I would not be able to complete my research project smoothly. I am also grateful to my classmates for lending me a helping hand whenever I need help and sharing useful opinions during this project.

Last but not least, I would like to express my deepest gratitude and appreciation to my supervisor, Dr. Ong Ghim Hock for his guidance and assistance throughout this research project. I truly appreciated his efforts and patience in correcting and improving my work throughout this journey.

ABSTRACT

Improper disposal and spillage of spent engine oil into the environment will lead to water and soil pollution. Environmental pollution is becoming a major worldwide problem as it will lead to adverse health effects in humans through the food chain. In order to overcome this problem, mycoremediation as an effective and inexpensive alternative is employed in cleaning up engine oil pollution. The first objective of this study was to screen for potential fungi in tolerating spent engine oil by using BHB as selective media. Fungi that were able to grow in BHB supplied with spent engine oil were identified as potential fungi in utilizing spent engine oil as carbon source. The second objective of this study was focused on determination of effectiveness of potential fungi in degrading spent engine oil via confirmatory test by calculating the percentage of degradation of spent engine oil. Six species of fungi namely *Mucor sp., Aspergillus flavus, Candida sp., Aspergillus niger, Aspergillus nidulans* and *Trichoderma sp.* were tested in this study. The results showed that *Mucor sp.* and *Trichoderma sp.* demonstrated high tolerance towards spent engine oil as indicated by its heavy growth rate, *Aspergillus nidulans, Aspergillus niger,* and *Candida sp.* showed moderate tolerance ability towards spent engine oil as denoted by its moderate growth rate whereas *Aspergillus flavus* was intolerant to spent engine oil as indicated by its absence of growth. In the confirmatory test, *Mucor sp.* (42.68%) was identified as the most effective fungi in degrading spent engine oil, followed by *Aspergillus nidulans* (39.32%), *Aspergillus niger* (37.42%), *Trichoderma sp.* (36.69%), *Candida sp.* (36.59%) while *Aspergillus flavus* (33.37%) was identified as the least effective fungi in degrading spent engine oil. Therefore, it was concluded that *Mucor sp.* was identified as the fungi with the most potential for application in mycoremediation of spent engine oil due to its high growth rate and highest percentage of degradation of spent engine oil as compared to other fungi species in this study.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADH	Alcohol Dehydrogenase
A. flavus	*Aspergillus flavus*
A. nidulans	*Aspergillus nidulans*
A. niger	*Aspergillus niger*
AGO	Automotive Gas Oil
BHB	Bushnell Haas Broth
d	day
°C	degree Celsius
g	gram
g/L	gram per litre
LAF	Laminar Air Flow
LSD	Least Significant Difference
µm	micrometer
mg/L	milligram per litre
mL	millilitre
min	minute
ANOVA	One-way Analysis of Variance
%	percent
PAHs	Polycyclic Aromatic Hydrocarbons
PDA	Potato Dextrose Agar
rpm	revolutions per minute
s	second
sp.	species
TPH	Total Petroleum Hydrocarbon

CHAPTER 1

INTRODUCTION

Engine oil which is derived from crude oil is widely used as a lubricant in automobiles as well as in maintenance of industrial generators for motor and internal combustion engines (Osaigbovo, Law-Ogbomo & Agele, 2012). Spent engine oil is produced after undergoing high temperature and tremendous mechanical pressure. In most of the countries, used engine oil is improperly disposed of by mechanics into open land, runoffs, drainages and gutters, contaminating water and soil. Besides that, engine oil is spilled from exhaust systems into the environment during engine operation due to leakage of engine and eventually contributes to water and soil pollution (Agarry & Ogunleye, 2012).

The composition of spent engine oil contains higher amount of polycyclic aromatic hydrocarbons (PAHs) and metals which are carcinogenic and mutagenic (Obayori, Salam & Ogunwumi, 2014). This can lead to long term damage in human health because the hazardous contents of engine oil may accumulate in plant tissues, organs of animals and humans, affecting the entire food chain. As a result, intoxication of various major organs may occur, leading to increased risk of cancer as well as other diseases (Mbachu, Chukwura & Mbachu, 2016). At present, engine oil pollution is becoming a major concern worldwide due to the increasing number of automotive vehicles and industrial machinery (Abioye, Agamuthu & Abdul Aziz, 2012). It is estimated that the loss of used engine oil to the environment across the globe is 2.7 billion gallons every 100 days (Biosynthetic Technologies, n.d.).

Various remediation technologies by chemical, mechanical and physical means have been employed in order to restore the hydrocarbon-polluted soil (Adekunle, Ani & Kanife, 2015). However, these technologies are expensive and not effective due to incomplete degradation of contaminants and less environmental-friendly because the indigenous organisms and nutritional value of soil will be destroyed as well (Thakur, 2014). Hence, mycoremediation which is the removal of pollutants by using fungi emerges as a cost-effective alternative (Hamba & Tamiru, 2016). Fungi show greater

potential in degrading a range of contaminants as compared to bacteria due to its special attributes, versatility and capability to survive in the stressed environment (Thenmozhi, Arumugam, Nagasathya, Thajuddin & Paneerselvam, 2013). Recent studies also show that secretion of extracellular enzymes and acids by mycelia of fungi are able to degrade hydrocarbons effectively (Adekunle, Ani & Kanife, 2015).

Based on the Environmental Quality Regulations in Malaysia, the content of oil in sewage and industrial effluent to be discharged into the environment must not exceed 10 mg/L (JAS, 2017). However, it is reported that a total of 121 cases of oil pollution happened in Malaysia from 2009 to 2015 (Fernando, 2016). Moreover, a major oil spill which resulted in the discharge of 300 tonnes of oil into Malaysian water in early 2017 raised concerns about the need to clean up the oil-polluted environment (Vanguard, 2017). Therefore, it is essential to identify the appropriate species of fungi to be used in order to degrade spent engine oil effectively.

The objectives of this study were:

1. To screen for potential fungi in tolerating spent engine oil.
2. To determine the effectiveness of the potential fungi in degrading spent engine oil via confirmatory test.

CHAPTER 2

LITERATURE REVIEW

2.1 ENGINE OIL

Engine oil which is also known as lubricating oil or motor oil is produced from base oil that is derived from petroleum distillation (Osaigbovo, Law-Ogbomo & Agele, 2013). It is considered as one of the most widely used products derived from the petroleum refining process due to its capability in lubricating engines in automobiles and power generating sets which is important to ensure smooth operation of an engine (Obayori, Salam & Ogunwumi, 2014). Engine oil mainly consists of a mixture of hydrocarbons which are aliphatic, aromatic, branched and some organometallic compounds as well as additives to improve its properties in which most of them are toxic to living organisms and environment (Mbachu, Chukwura & Mbachu, 2016).

Classification of engine oil can be done according to their composition, chemical structure, mode of application and molecular weight. Generally, engine oil is divided into mineral oil based and synthetic in which the mineral oil based engine oil is refined from crude oil whereas the synthetic engine oil is chemically synthesized from hydrocarbon compounds (Omoghie, Udochukwu & Oshionebo, 2014). The composition of engine oil will be modified after subjecting to high pressure and high temperature during engine operation. Spent engine oil which is a brown and black liquid will be produced and drained out from industrial and automobile engines during manual changing of oil in engine servicing (Agarry & Ogunleye, 2012).

2.1.1 Composition of Engine Oil

Research has shown that the composition of fresh and spent engine oil are different in terms of hydrocarbon structure, chemical content and metal content. Fresh engine oil contains 99.7% of aliphatic hydrocarbon, 0.27% of aromatic hydrocarbon and 0.03% of olefinic hydrocarbon whereas spent engine oil contains 98.9% of aliphatic hydrocarbon, 0.94% of aromatic hydrocarbon and 0.08% of olefinic hydrocarbon. The significant increase of aromatic hydrocarbon content in spent engine oil is due to oxidation of engine oil during motor operation (Kupareva et al., 2013).

Another significant difference between fresh and spent engine oil is the content of polycyclic aromatic hydrocarbons (PAHs) and metals which are harmful to living organisms. The amount of PAHs such as naphthalene and alkyl benzene in spent engine oil is higher when compared to fresh engine oil with relatively low amount of PAHs. This is because more PAHs will be synthesized via pyrosynthesis as engine oil is exposed to high temperature during engine operation. Besides that, spent engine oil contains higher concentration of additional chemicals due to breakdown of additives (Samuel, 2011). In addition, metals such as chromium, zinc and copper are produced in spent engine oil when engine parts wear down during engine operation (Obayori, Salam & Ogunwumi, 2014).

2.1.2 Spent Engine Oil as Nonpoint Source of Oil Pollution

Improper disposal of spent engine oil is one of the major nonpoint source of oil pollution across the globe, especially in the developing countries. Only one liter of engine oil is sufficient to cause the pollution of a million gallons of water (Samuel, 2011). It is reported that 363 million gallons of spent engine oil enter waterways and eventually end up in the oceans worldwide every year (Clayton, 2010). This is because used engine oil is directly discharged into open ground, gutter and drainages by mechanics and end up in runoff water. Besides, the spillage of spent engine oil from exhaust system into the open space due to engine leakage also contributes to contamination of runoff water (Agarry & Ogunleye, 2012). As a result, runoff water that is polluted by spent engine oil will flow into soil and waterways like rivers, lakes and oceans which results in pollution of water, soil and groundwater (NOAA, 2008).

Due to the increasing use of engine oil in automobiles and industry worldwide, engine oil pollution which is a nonpoint source pollution is receiving more attention in urban area as compared to crude oil pollution (Abioye, Agamuthu & Abdul Aziz, 2012). In addition, it is also more difficult to be controlled compared to crude oil pollution because it is caused by pollutants originating from multiple different places (NOAA, 2008). Although it is possible to recycle spent engine oil to produce re-refined engine oil, it is only done by the developed countries like United States where only 10-15% of spent engine oil is re-refined (Handwerk, 2011).

2.1.3 Impacts of Engine Oil Pollution

Spent engine oil that is released into the environment can threaten living organisms including animals, plants and humans. The presence of hazardous compounds such as PAHs and metals in spent engine oil will cause acute and chronic toxicity, mutagenicity and even carcinogenicity in living organisms (Samuel, 2011). Accumulation of fat soluble contents of spent engine oil in plant tissues as well as animal and human organs will cause the whole food chain to be affected. Intoxication of major organs like kidney and liver, reproduction problems as well as various types of cancer and diseases may develop due to long term exposure to the hazardous contents in spent engine oil (Mbachu, Chukwura & Mbachu, 2016). Besides that, developmental problems and unpleasant changes in anatomical characteristics of human may also occur (Obayori, Salam & Ogunwumi, 2014).

On the other hand, inappropriate disposal of spent engine oil into open spaces will cause soil and water pollution. Hazardous compounds such as PAHs which are highly recalcitrant will persist in the soil and not easily degraded due to its low water solubility (Okparanma, Ayotamuno, Davis & Allagoa, 2011). The soil condition will no longer be suitable for growth of plants due to the formation of oily scum that impedes availability of oxygen, nutrients and water to plant roots. Therefore, the growth of plants will be stunted and this will lead to low yield of crops in agriculture (Osaigbovo, Law-Ogbomo & Agele, 2013). In addition, metabolism of plants and growth of soil organisms like fungi and bacteria will be influenced due to the toxicity of the compounds present in the spent engine oil (Obayori, Salam & Ogunwumi, 2014). Besides, water pollution that caused by the entry of spent engine oil into different