USAGE OF FUNGI IN HEXADECANE TOLERANCE

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ABSTRACT

Abundantly used of hexadecane in the production of fuel oil has led to the accumulation of the pollutant in soil and water which result in serious impacts on human health. In order to overcome this problem, mycoremediation approach has become the choice to remove hexadecane from polluted environment. Mycoremediation provides effective clearance of hexadecane using fungi and it was cost effective. This main objective of this study is to determine the ability of different species of fungi tolerating hexadecane by using BHB medium which carbon source was absence. Fungi species namely *Aspergillus nidulas, Mucor sp, Aspergillus flavus, Aspergillus niger, Trichoderma sp and Candida sp* had been screened out as the potential fungi in tolerating hexadecane. Then the screened out fungi were proceed to test on their ability in hexadecane tolerance by measuring their dry weight. The higher the dry weight obtained, the higher the potential to be hexadecane tolerance fungi. *Mucor sp.* (0.011 g) showed the highest tolerance towards hexadecane, followed by *Trichoderma* (0.007 g). *Candida* species (0.005 g) showed average tolerant capability to hexadecane. However *Aspergillus* genera such as *Aspergillus niger* (0.001 g) showed lower tolerance ability in comparison with the screened out potential fungi on hexadecane. Therefore in this experiment, *Mucor sp.* was found to be the most potential species to be used in mycoremediation for hexadecane when compared to others tested fungi.

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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
ANOVA	Analysis of Variance
BHA	Bushnell Haas Agar
BHB	Bushnell Haas broth
°C	Degree Celsius
g	gram
LAF	Laminar Air Flow
LSD	Least significant different
L	Liter
mL	Millimeter
mins	minutes
%	percentage
PDA	Potato dextrose Agar
rt	Room temperature
sp	species
SPSS	Statistical Package of the Social Sciences

Chapter 1.0

INTRODUCTION

Hydrocarbon can be categorized into four main types which are aromatic, resin (pyrimidines), the aliphatic and lastly asphaltenes (fatty acids, ester and phenols) (Das, Chandran, Das, & Chandran, 2011). Aliphatic hydrocarbon especially the medium chain size hydrocarbon such as hexadecane is the major soil pollutant (Dehghani, Taatizadeh, & Samaei, 2013).

Aliphatic hydrocarbon can be found mostly in crude oil which mainly acts as surrogate component in petroleum and diesel (Palm, 2009). Due to high demand of petroleum from the public, it has contributed to the increase of hexadecane release to the environment. The leakage of organic based hydrocarbon might cause ecosystem imbalance in the sense that toxic related products released into the environment caused the death of animals and plants (Chikere & Azubuike, 2014).

Therefore, many technologies have been applied in soil remediation such as chemical and physical practices. These methods are not effective because incomplete decomposition of contaminants happened (Das et al., 2011) and it might cause human health is at risk (Yang, Ligang, An, & Beihong, 2015). As a result, more attentions have been given on using microorganism to remove the pollutant which is also known as bioremediation. This approach provide eco-friendly and cost effective result (Morales, 2014) in comparison to other methods. The used of fungi for remediation also known as mycoremediation has been widely study due to fungi specific properties such as the ability to survive in harsh conditions (Ekundayo & Osunla, 2013).

Fungi are widely used to clean environmental pollutants not only hydrocarbon but also heavy metals as fungi are unique equipped for remediation (Magdalene &

Ariyo, 2009). It has been reported that fungi have the ability to fully degrade petroleum pollutant in soil environment (Makut, Ogbonna, Ogbonna, & Wuna, 2014). Hence, this experiment comes to play whereby potent fungi such as *Aspergillus nidulus. Mucor,* Aspergillus flavus, *Aspergillus niger, Trichoderma sp*, Candida *sp* are used in mycoremediation provide high efficiency in the clearance of pollutants.

The objectives of this research were:

1. To screen for the potential fungi in tolerating hexadecane.
2. To identify the tolerance ability among the screened out fungi.

CHAPTER 2.0

LITERATURE REVIEW

2.1 HEXADECANE

Hexadecane is frequently selected in many research project due to its low water solubility properties and it tend to be degraded rapidly by variety types of microorganism (Dehghani et al., 2013). Hexadecane is a hydrocarbon compound which is classified in the alkane family with the formula $C_{16}H_{34}$ (Poyntner, 2014). This compound is made up of 16 carbons with three hydrogen atoms are bonded to each end of the carbon. The remaining carbon atoms bonds to two hydrogen bonds. Figure 2.1 below shows the structure of hexadecane.

Figure 2.1 The chemical structure of hexadecane (Poyntner, 2014).

Hexadecane is considered as an important component in petroleum such as diesel and kerosene. It has been reported that hexadecane can also be found in rubber, biomass and even in tobacco smoke (Poyntner, 2014). To date, hexadecane has become a typical pollution issue because of industrial and domestic extensive usage and their irresponsible disposal behaviour (Morales, 2014).

The pollution caused by hexadecane has become a big concern to the society because hydrocarbons are toxic to all organisms (Abha & Singh, 2012). For example, prolong inhaling of volatile aliphatic hydrocarbon can cause the person suffer from respiration impaired (Von Oettingen, 1942). Besides, the typical symptoms such as dryness and cracking of skin can be observed and it might cause death when hexadecane is ingested (Dehghani et al., 2013). According to Law and his colleagues, Malaysia water and sediment are generally considered safe provided that hydrocarbon LC_{50}

concentration should be less than 100 μg L^{-1} and 100 mg kg^{-1} respectively. (Law & Hii, 2006).

Therefore, plenty of methods have been proposed to remove hexadecane. However, the biological method is performed due to its lower cost as well as environmental friendly properties (D'Annibale, Rosetto, Leonardi, Federici, & Petruccioli, 2006).

2.2 BIOREMEDIATION

Due to globalisation, releasing of chemicals from industry caused petroleum contamination (Battelle, 2007) leading to severe pollution in the environment. In order to clean the pollutants, chemical remediation, physical remediation, bioremediation are the common methods can be used to control and remove the pollution level in the environment (Yang et al., 2015).

However several problems have arisen by using chemical remediation and physical remediation (Dehghani et al., 2013) even though it has rapid cleaning effect in comparison to bioremediation (Abha & Singh, 2012). Dumping chemicals to the pollutant sites or using high temperature to degrade pollutants have become the limitation when chemical and physical methods are used (Rhodes, 2014). These methods that used to clean up the pollutants tend to be expensive and generate further pollution such as global warming (Morales, 2014) . Therefore, bioremediation has attracted worldwide attention.

Green remediation including bioremediation is a clean-up action using the best remedy to restore the polluted sites to a clean sites with a minimal amount of associated costs (USEPA, 2008). Until now, bioremediation provides rapid treatment and overall high efficiency result compared to conventional methods (Abha & Singh, 2012). Besides, bioremediation has become a hot solution to treat the pollutants such as hydrocarbons because microbes have enzyme systems to degrade the hydrocarbon and utilized the products (carbon) as a source of energy (Das et al., 2011). Table 1 shows microbial remediation has higher efficiency compared to others technology listed in Table 2.1.

Table 2.1 Evaluation of the remediation technology of polluted farmland (Yang et al., 2015).

Technologies	Remediation efficiency, %	Cost
Replacing soil	> 95	Moderate
Physical separation	50-90	Low
In situ/ ectopic fixed	> 90	Moderate
Electric heating	>90	Moderate
Vitrification	>90	High
Soil- vapour-extraction	75-90	Low
Electrokinetic remediation	50-90	High
Soil washing	>90	Moderate
In situ chemical oxidation	>50	Moderate
In situ chemical reduction	>50	Moderate
Phytoremediation	<75	Low
Microbial remediation	70-90	Low
Biological compost	>75	Low
bioventing	>90	Low
bioreactor	>90	Low to moderate

Microbial remediation, phytoremediation, and mycoremediation are three main popular types of bioremediation that have been used widely in this current society. In this project, my research study is focus on mycoremediation.

2.2.1 Mycoremediation

In mycoremediation, fungi are useful in degrading complex biomass and xenobiotics (Bianco & Perrotta, 2015) and their high ability in hydrocarbon degradation properties ranged from 6% to 82% (Das et al., 2011). Fungi have been chosen because of their special features such as ability to survive in stress conditions and its rapid growth characteristics (Chikere & Azubuike, 2014). Fungi tend to have high sexual reproduction under high stress condition and thus arise new species of fungi species for hydrocarbon degradation activity posed by human irresponsible deeds (Norton, 2012).

Besides, fungi are less sensitive to the physical changes in pH, temperature, and aeriation (Gupta, 2014). Therefore, they are able to show good tolerance and survival in harsh conditions. According to Hughes and his colleague, filamentous fungi are tends to find aliphatic polluted area (Hughes, Bridge, & Clark, 2007). This is because filamentous fungi can detoxify and degrade toxic pollutant but also concentrate and stored toxic heavy metals by forming fruiting body (Beukeboom, n.d.).

In biomass degradation, fungi species produced different enzyme such as cellulases, hemicellulases, and ligninases. Brown rot fungi are able to hydrolyse cellulose and hemicelluloses by cellulose enzyme to obtain carbon sources for their growth, however, white rot fungi are responsible for attacking lignin in biomass (Bianco & Perrotta, 2015) by secreting lignin peroxidase. Other enzyme such as alkane monoxygenase also secreted by fungi to degrade hexadecane (Singh, 2006). The degradation mechanism is discussed on topic 2.2.3. In brief, enzymes that produced by fungi are important in degrading biomass in the environment (Dashtban, Schraft, & Qin, 2009).

Besides, certain species of fungi are able to synthesize biosurfactant which can increase the solubility of insoluble hydrocarbon or partial soluble hydrocarbon (Silva et al., 2014) by decreasing the interfacial surface tension between liquid and hydrocarbon polymer thus it is more effective in stabilizing oil-water emulsion (Rodrigues & Keller, 2015). As a result, biosurfactant has increased the bioavailability of the hydrophobic pollutant enabling the fungi to degrade hydrocarbon more effectively (Das et al., 2011). Biosurfactant has been favour by many industry because it is eco-friendly and cost effective for bioremediation (Bhardwaj, 2013).

However, some of the fungi had the ability in storing hexadecane instead of degrading it. Fungi might absorbed alkane into hydrophobic compartment of the cell wall and stored as lipid bodies. Fungi cell wall was made up of framework of polysaccharide and cell wall protein was found in the cell (Pitarch, Nombela, & Gil, 2008) which result in the formation of hydrophobic compartment. One of the article have reported that lipid bodies were found in yeast cell when treated in glucose medium (Fickers et al., 2005). Hence, the potential fungi might tolerance to hydrocarbon by absorbing the alkane into hydrophobic compartment of cell wall.