THE GROWTH AND LACCASE ACTIVITY OF EDIBLE MUSHROOMS IN PLASTICS DEGRADATION

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A. bisporus	*Agaricus bisporus*
BPA	Bisphenol A
BHB	Bushnell Haas Broth
°C	Celcius
HDPE	High-Density Polyethylene
IU	International Units
LiP	Lignin Peroxidase
LDPE	Low-Density Polyethylene
MnP	Manganese Peroxidase
mg/L	Milligram Per Litres
mL	Millilitres
mM	Millimolar
M	Molar
P. abalones	*Pleurotus abalones*
P. ostreatus	*Pleurotus ostreatus*
PE	Polyethylene
PS	Polystyrene
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
rpm	Revolutions Per Minutes
NaCl	Sodium Chloride
UV	Ultraviolet

CHAPTER 1:

INTRODUCTION

Plastics bring lots of benefits and harms to human and environment. Plastics have been widely used and produced in large scale since 1950s. There are different types of plastics materials such as low-density polyethylene (LDPE) and polystyrene which could be found in our daily life usage like plastics bag or food container (Siddiqui, Gondal & Redhwi, 2008). Plastics contains plasticizer such as Bisphenol A (BPA) or phthalate which is potential harmful to human health as both BPA and phthalate can be function as endocrine disrupting agents (North & Halden, 2013).

According to Geyer, Jambeck and Law (2017), methods to reduce plastics wastes include recycling, burning or accumulating in landfill. But according to the statistic, only 9% of the plastics is being recycles while the rest is either burnt or accumulated in landfill. These methods brought side effects to the environment like air and water pollution (North & Halden, 2013). Other more environment friendly methods include replacing the current plastics material with a more degradable plastics material like cellulose acetate (Hopewell, Dvorak & Kosior, 2009) or by limiting the usage of plastics (Caruso, 2015) to reduce the plastics pollution in the environment.

Mycoremediation techniques had been introduced to degrade plastics by using fungi (Zhang, Li, Chen & Li, 2015). Most of the potential fungal species are belong to the phylum of Ascomycetes, Basidiomycetes and Deuteromycetes (Brijwani, Rigdon & Vadlani, 2010). Some of the examples of fungi used are *Aspergillus niger*, *Pleurotus ostreatus*, *Ganoderma lucidum* (Muthukumar & Veerappapillai, 2015). Sometimes, edible mushrooms are used as they require less cost, available in large amount (Valverde, Hernández-Pérez & Paredes-López, 2015), had been widely studied (Magan, Fragoeiro & Bastos, 2010) and are more environment friendly as it does not involve any chemical (El-Morsy, Hassan & Ahmed, 2017).

According to a study carried out by Zhang et al. (2015), fungi can produce an extracellular enzyme called laccase that is responsible to degrade plastics. Besides that,

it can also be used to degrade xenobiotics, to decolorize dye and to treat effluent (Viswanath, Rajesh, Janardhan, Kumar & Narasimha, 2014). Plastics degradation depends on factors like the properties of plastics (Tokiwa, Calabia, Ugwu & Aiba, 2009) and environmental factors (Kapahi & Sachdeva, 2017) like pH, temperature, salinity and nutrients availability (Flashinski & Lichtensein, 1975). As fungi require specific condition to have optimum grow, thus it is important to know their optimum range to grow and survive in different environmental condition (Muthukumar & Veerappapillai, 2015).

In this experiment, tissue culture technique was used to cultivate mushrooms namely *Pleurotus ostreatus*, *Pleurotus abalones* and *Agaricus bisporus* which are available in large amount (Valverde et al., 2015) and can be easily cultivated on PDA media (Sánchez, 2010). With the increasing number of plastics in rivers and increasing temperature (Hammer, Kraak & Parson, 2012), it is important to determine the optimum temperature and salinity level that a mushroom is able to tolerate and undertake mycoremediation.

Thus, the objective of my study was to

i. Determine the optimum level of different temperature and salinity level on enzymes laccase activity and growth of edible mushrooms.
ii. Determine laccase enzymes activity and growth of edible mushrooms during plastics degradation.

CHAPTER 2:

LITERATURE REVIEW

2.1 FUNGAL AS A BIOREMEDIATION TOOLS

2.1.1 Mycoremediation

Mycoremediation is a process where edible or non-edible fungi is used to degrade and transform different kind of industrial waste into a useful and safer product. There are currently three main methods to clean up the polluted site using fungi which is through biodegradation, biosorption and bioconversion (Kulshreshtha, Mathur, & Bhatnagar, 2014). Most of the mushrooms species are able to degrade polymers like plastics (da Luz et al., 2013) or polyaromatic hydrocarbon (PAH) (Prakash, 2017) by oxidation process. Biosorption method is mostly used to treat heavy metal in polluted site, where dried or live mushrooms can be used to absorb heavy metal into their fruiting body or mycelium (Kapahi, & Sachdeva, 2017). Bioconversion process is a method where industrial wastes are used as a substrate to cultivate mushrooms, which this will eventually recycling and reuse the wastes produced (Kulshreshtha et al., 2014).

2.1.2 Edible Mushroom Species

Edible mushrooms had been consumed since ancient Greeks period. They believed that mushrooms can be used to enhance strength, improve health and use it as a culinary (Valverde et al., 2015). As mushrooms have been proven to contains lots of nutrients, thus, it had been mass cultivated and produced worldwide especially in China. Some of the most cultivated species are *Agaricus bisporus*, *Pleurotus abalones* and *Pleurotus ostreatus* (Valverde et al., 2015) as shown in Figure 2.1 (a), 2.1 (b) and 2.1 (c). These white rot fungi had been widely studied since 1980s to prove its ability on degrading various types of xenobiotics (Magan et al., 2010).

(a) (b) (c)

Figure 2.1 (a) The common cultivated mushrooms species, *Pleurotus ostreatus*; **(b)** *Agaricus bisporus* (Wikimedia, 2009) and **(c)***Pleurotus abalones* (SpecialtyProduces, n.d).

2.2 EXTRACELLULAR ENZYMES

Fungi are able to produce various types of extracellular enzymes like laccase, manganese peroxidase (MnP), lignin peroxidase, cellulase, amylase (da Luz, Paes, Nunes, da Silva & Kasuya, 2013), esterase, lipases and cutinase (Ojha et al., 2017) that are responsible to degrade different types of xenobiotics (Dashtban, Schraft, Syed & Qin, 2010).

Laccase is a blue multi-copper oxidase that able to oxidize (Upadhyay, Shrivastava & Agrawal, 2016) different aromatic or non-aromatic compounds (Claus, 2004). This enzyme can be found abundantly in fungi especially white-rot fungi (Viswanath et al., 2014). Whenever laccase is used to degrade polymers like plastics component or PAHs, it will activate radical-catalyzed reaction mechanisms. This mechanism can cleave the covalent bonds between polymers and lead to the formation of monomers that is more stable, safe and small (Claus, 2004). Study conducted by Viswanath et al. (2014) stated that production of this enzymes will be affected by different abiotic factors like temperature, pH, nutrients availability and more. The discovery of laccase leads to the massive usage in biotechnology fields to degrade dye, degrade toxic wastes, treat soil, develop biosensor, use in medical and food products (Upadhyay et al., 2016), synthesize gold nanoparticle (El-Batal, ElKenawy, Yassin & Amin, 2015) or degrade plastics components (Zhang et al., 2015).

2.2.1 Laccase Enzymes Assay

There are several methods to detect the presences of laccase enzymes which by using different substrate like ABTS (More, PS & Malini, 2011), 2, 6-DMP (Heinzkill, Bech, Halkier, Schneider & Anke, 1998), syringaldazine (Dedeyan et al., 2000) and guaiacol (El Monssef, Hassan & Ramadan, 2016). All of these substrates can be oxidized by laccase to form a different color. For example, ABTS formed blue-green color (More et al., 2011), 2, 6-DMP formed orange and reddish in color, syringaldazine formed a color of pink or purple (Harkin, Larsen & Obst, 1974) while guaiacol formed a reddish-brown color (El Monssef et al., 2016).

2.2.2 Other Extracellular Enzymes

Manganese peroxidase (MnP) is an extracellular enzyme (Dashtban et al., 2010) that able to oxidize Mn^{2+} to Mn^{3+} (Sayadi & Ellouz, 1995). This enzyme can be found abundantly in basidiomycetes such as *Panus tigrinus* and *Agaricus bisporus* (Dashtban et al., 2010). This enzyme can be used to degrade phenolic lignin in pulp (Dashtban et al., 2010), dyes or other non-phenolic compounds (Coconi-Linares et al., 2014).

Lignin peroxidase (LiP) is another type of extracellular enzyme (Falade et al., 2017) that able to oxidize and cleaves ether or two carbon bonds in non-phenolic aromatic substrates (Coconi-Linares et al., 2014) or phenolic compounds (Falade et al., 2017). This enzyme can be found abundantly in white rot fungi like *P. chrysosporium* and *T. versicolor* (Dashtban et al., 2010). This enzyme can be used to degrade recalcitrant polymer lignin, degrade dye and more (Patil, 2014).

2.3 FACTORS AFFECTING LACCASE ENZYMES ACTIVITY

2.3.1 Temperature

Temperature had been one of the factors that will affect the rate of biodegradation, fungal growth and enzymes activity. Brijwani et al. (2010) stated that the optimum temperature for fungal to produce laccase is around 25 to 30°C and the production will be lower when the temperature is going higher than 30°C due to the growth of fungal is

inhibited (Patrick, Mtui, Mshandete & Kivaisi, 2011). Ilyas and Rehman (2013) showed that most of the laccase enzyme produced by fungal able to work well at temperature of 40 to 50°C. On the other hands, Šnajdr and Baldrian (2007) found out that *Pleurotus ostreatus* can grow well and produces both MnP and laccase in highest amount when the temperature reaches 25 to 30°C. They also stated that the optimum temperature of laccase enzyme activity is around 50°C while MnP is around 60°C.

2.3.2 pH

The pH level is also another factor that will affect the enzymes activity. Brijwani et al. (2010) stated that the best pH level for fungal to produces enzymes is around 4.5 to 6.0. While there is another report stated by Singh (2006) that fungi can grow well when the pH value is about 4 to 5. According to research done by Zeng, Zhao and Xia (2017), they found out that the optimum pH range of fungi on degrading BPA chemicals is around 5. Research carried out by Patrick et al. (2011) also stated that fungal enzymes will have the highest activities when the pH is ranged 4 to 6, they also find out that pH 3.5 will has a lower enzymatic activity on both MnP and laccase due to the acidic condition. This factor is not study as water from ocean or river normally maintained at pH of 6.0 to 8.0 (WaterQuality, n.d.).

2.3.3 Salinity Level

Salinity level is another environmental factor that will affect the biodegradation of plastics (Olaosebikan Oluwatosin, Alo, & Ugah, 2014). Study conducted by Zilly et al. (2011) show that the presences of NaCl will lower down the laccase enzyme activity even when it is in low concentration like 0.4 to 1.0 M, but laccase enzyme activity is slightly active when Na_2SO_4 was added. Study conducted by D'Souza-Ticlo, Garg and Raghukumar (2009) also stated that addition of NaCl will inhibit the fungal laccase activity and 0.1% of NaCl will be the best for fungal laccase activity and its growth.