

DST 001345

TAN SRI ABDUL MAJID LIBRARY

EFFECT OF CHROMIUM AND ALUMINIUM ON
THE GROWTH OF *Pleurotus ostreatus*

WEE ING KIAN

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

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Dr. Thong Weng Hing.

Wee Ing Kian

Student ID: I14006010

Date: 07/05/2017.

Dr. Thong Weng Hing

(SUPERVISOR)

ACKNOWLEDGEMENT

First and foremost, I would be obliged to the Almighty God for establishing me to complete my final project. Throughout the 10 weeks of laboratory work, I faced unexpected hardships and problems in my research. I am grateful to my supervisor who gave me valuable guidance and expertise from the beginning until the end to complete my final project. Then I would like to express my sincere acknowledgement to INTI University, Nilai campus that allowed me to precede my final project successfully by providing the proper laboratory, materials and advanced equipment. Last but not least, I would like to send my sincere appreciations to my family and friends for their invaluable assistance and encouragements during my final project period.

ABSTRACT

Pollution of heavy metal was concerned as a critical and crucial issue with regards to environment biotechnology and agriculture biotechnology. A high concentration of heavy metal had been acknowledged as toxic for all organisms. In this case, toxicity of chromium would inhibit ion of enzymatic activity, carcinogenic and so on. However, due to being the highest contribution in earth crust, aluminium (Al) had become another main study for pollution problem. Therefore for my study, mycoremediation would be used to reduce the heavy metal contamination from soil and environment. Therefore, *P. ostreatus* was decided to be used to remove the heavy metal. The focus of my study was to determine the effect of chromium and aluminium on the growth of *P. ostreatus* as well as the effect of pH values on the growth of *P. ostreatus*. In this case, the mycelium of *P. ostreatus* would be cultured on potato dextrose agar (PDA) medium and the parameters involved were the different concentrations of Aluminium(Al) and Chromium (Cr) in different pH values. The optimum Cr and Al concentration for growth of *P. ostreatus* were found to be 40 mg/L and 120 mg/L respectively. On the other hand, the optimum pH value for growth of *P. ostreatus* in this research was pH 7 and pH 6 for both Cr and Al supplemented PDA media.

TABLE OF CONTENT

	Page
NON-PLAGIARISM DECLARATION	ii
DECLARATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
TABLE OF CONTENT	vi
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATION	x
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	3
2.1 Heavy Metal Pollution	3
2.2 Chromium Toxicity	3
2.3 Aluminium Toxicity	4
2.4 Biosorption	5
2.5 Mycoremediation	6
2.6 <i>Pleurotus ostreatus</i>	6
2.6.1 Adsorption of Cr(VI) and Al in <i>Pleurotus ostreatus</i>	7
2.6.2 Optimum pH for adsorption of Cr(VI) and Al for <i>Pleurotus ostreatus</i>	7
2.7 Potato Dextrose Agar (PDA)	7
3 MATERIALS AND METHODS	9
3.1 Culturing Material	9
3.2 PDA Medium Preparation	9
3.3 Cr(VI) Supplemental PDA medium Preparation	9
3.4 Al Supplemental PDA preparation	10
3.5 <i>Pluerotus ostreatus</i> cultivation in PDA medium containing different concentration of Cr or Al	10
3.6 <i>Pleurotus ostreatus</i> Cultivation in PDA Medium Containing 80 mg/L Concentration of Cr or Al with Varied pH	11
3.7 Statistical Analysis	11
4 RESULTS	12
4.1 Effect of Difference Concentration of Cr on the Growth of <i>Pleurotus ostreatus</i>	12

4.2	Effect of Difference Concentration of Al on the Growth of <i>Pleurotus ostreatus</i>	14
4.3	Comparison of Effect of Cr and Al on the growth of <i>Pleurotus ostreatus</i>	16
4.4	Effect of Different pH in Cr Supplemented PDA Media on the Growth of <i>Pleurotus ostreatus</i>	17
4.5	Effect of Different pH in Al supplemented PDA Media on the Growth of <i>Pleurotus ostreatus</i>	19
4.6	Comparison of pH in Cr and Al supplemented PDA Media in the Growth of <i>Pleurotus ostreatus</i>	20
5	DISCUSSION	22
5.1	Effect of Al and Cr in Growth of <i>Pleurotus ostreatus</i>	22
5.2	Heavy metal tolerance of <i>Pleurotus ostreatus</i> in Cr and Al supplemented PDA Media	22
5.3	Effect of pH in the Cr Supplemented PDA Media	23
5.4	Effect of pH in the Al Supplemented PDA Media	23
5.5	Contamination Species in Al Treatment Agar	24
6	CONCLUSIONS AND RECOMMENDATION	25
	REFERENCES	26
	APPENDIX	33

LIST OF TABLES

Table		Page
3.1	Volume of Cr stock solution and deionised water added in total volume of 400 mL	9
3.2	Volume of Al stock solution and deionised water added in total volume of 400 mL	10

LIST OF FIGURES

Figure		Page
1	Mycelium of <i>P. ostreatus</i> produced in PDA medium on day 5.	12
2	Mycelium growth of <i>P. ostreatus</i> on day 5 in (A) 5 mg/L Cr, (B) 10 mg/L Cr, (C) 20 mg/L, (D) 40 mg/L. (E) 80 mg/L. (F) 120 mg/L.	12
3	Surface area coverage of <i>P. ostreatus</i> mycelium under different concentration of Cr on day 5 .	13
4	Mycelium growth of <i>P. ostreatus</i> on day 5 in (A) 5 mg/L Al, (B) 10 mg/L Al, (C) 20 mg/L Al, (D) 40 mg/L Al, (E) 80 mg/L Al, (F) 120 mg/L Al.	15
5	Surface area coverage of <i>P. ostreatus</i> mycelium under different concentration of Al on day 5 .	16
6	Comparison of surface area coverage in Cr and Al on day 5 .	17
7	Mycelium growth of <i>P. ostreatus</i> in 80 mg/L Cr on day 5 in (A) pH 5.6, (B) pH 6.5, (C) pH 7, (D) pH 8, (E) pH 9	18
8	Surface area coverage of <i>P. ostreatus</i> mycelium in 80 mg/L Cr supplemented media under different pH values on day 5.	19
9	Mycelium growth of <i>P. ostreatus</i> in 80 mg/L Al on day 5 in (A) pH 5, (B) pH 6, (C) pH 6.5, (D) pH 7, (E) pH 8, (F) pH 9.	20
10	Surface area coverage of <i>P. ostreatus</i> mycelium in 80 mg/L Al supplemented media under different pH values on day 5.	21

LIST OF ABBREVIATIONS

°C	Degree Celsius: Unit of Temperature
<i>P.ostreatus</i>	<i>Pleurotus ostreatus</i>
ANOVA	Analysis of Variance
Cr	Chromium
Al	Aluminium
%	Percentage
mgg ⁻¹	Milligram per Gram: Unit of concentration
g	gram: Unit of concentration
mg	Miligram: Unit of concentration
mgL ⁻¹	Miligram per Liter : Unit of concentration
mgmL ⁻¹	Miligram per Mililiter : Unit of concentration
mL	Mililiter: Unit of Volume
kPal	Kilopascal
Min	Minute
HCl	Hydrochloric acid

NaOH	Sodium Hydroxide
NaCl	Sodium chlorite
PDA	Potato Dextrose Agar
MT	Metallothionein
PCs	Phytochelatin
GSH	Glutathione
H_3O^+	Oxonium
$Al(OH)_3$	Aluminium Hydroxide
$Al(OH)^{2+}$	Aluminium monohydroxide ions
$Al(OH)_3^{4-}$	Aluminium hydroxide ions
pH	power of hydrogen: Measure of concentration of hydrogen ion

CHAPTER 1

INTRODUCTION

Pollution is defined as “the introduction of any kind of substance or energy to the surrounding or environment at a rate faster than the environment can convert it to certain harmless form” (“pollution | environment”, 2015). There are many kinds of pollutants and they exist in solid, liquid or gaseous form (“Environmental Pollution, Its Sources and Effects”, 2016). The common air pollutants are carbon monoxide, airborne articles, radioactive pollutants and so on (“Environmental Pollution, Its Sources and Effects”, 2016). On the other hand, contaminated water always contains detergent, food processing chemicals, and petroleum hydrocarbons. Besides that, heavy metal and hydrocarbons are some of the contaminants that pollute the soil. In some cases, some of the pollutants, for instance, pesticide, can be found in water, soil or air (Ali, Khan & Sajad, 2013).

Nowadays, pollution of heavy metal has become an important and crucial issue in the context of environment biotechnology and agriculture biotechnology. This is because the growth of plants always influence by the existence and concentration of heavy metal in the soil. Among these heavy metals, Cr is utilised as a part of metal compounds, for example, metal-pottery, stainless steel, and it is also used as chrome plating. Since Cr contribute a lot of benefit in industry, such as utilised to gives a solid, profoundly rust proof covering for heavy applications (“Chromium and its negative effects on the environment”, 2016). Since there is a range of Cr in the context of industry, the pollution of Cr had been concerned and studied. It is found that Cr toxicity in organisms include hindrance of enzymatic activities, nutrient and oxidative imbalances, carcinogenic and so on (Oliveira, 2012).

Aluminium, another heavy metal that is highly concerned because Al is the most broadly spread element around the planet and it is widely utilised as cookware, jars, aluminium foil and so on (“Why I'm Concerned About the Dangers of Aluminum”, 2012). In fact, accumulation of Al in the kidneys, cerebrum, lungs, liver and thyroid can influence skeletal mineralization (Kumar & Gill, 2009). For the influence in plant,

the accumulation of Al will inhibit the enzymatic activities and changes the permeability of cell membranes (Exley, Swarbrick, Gherardi & Authier, 2009).

Bioremediation has been implemented widely as an approach to solve the environmental pollution problem. Some of the industries have chosen bioaccumulation which is a type of bioremediation, to reduce the soil pollution. Bioaccumulation works on the principle to accumulate the pollutants such as heavy metal and pesticides into an organism X (Senadheera & Pathiratne, 2006). In this context, the ability to tolerate and accumulate heavy metal in fungus is basically higher than plants, therefore fungi is a potential and ideal organism for bioaccumulation (Senadheera & Pathiratne, 2006). Based on its high metal binding capacity and high tolerance to heavy metals, *Peurotus ostreatus*, also known as oyster mushroom can be used as a potential agent of mycoremediation (Akpaja, 2012).

Therefore, the objectives of this study were

1. to determine the effect of chromium and aluminium on the growth of *P. ostreatus*.
2. to determine the effect of pH values on the growth of *P. ostreatus* in Cr and Al supplemented media.

CHAPTER 2

LITERATURE REVIEW

2.1 HEAVY METAL POLLUTION

Heavy metals have been acknowledged as an essential trace elements for most of the organisms (Khan, 2011). The features of all heavy metal are that they contain a higher molecular weight and a fivefold density to water (Tchounwou, Yedjou, Patlolla & Sutton, 2012). Some heavy metals are present in natural environment as a toxic form (Alkorta et al., 2004). It is different from organic pollutants; the heavy metals are not biodegradable and they will accumulate in natural environment which causes various pollutions, namely air pollution, water pollution and soils pollution (He, Yang & Stoffella, 2005). In this case, a little amount of heavy metals had been reported to contribute to high toxicity and side effects to organisms (Martin, 1993). The toxicity of heavy metals depends on a number of factors involving the chemical species, dosage, route of exposure, gender, age and genetics (Bryan, 1957). Some of the heavy metals concerned are Cr, Pd, Hg and As. These heavy metals were considered systemic toxicants because of damaging organ in a lower levels of expose (Brookes, 1995).

2.2 CHROMIUM TOXICITY

The toxicity of Cr is due to its water-solubility and oxidation state ("Chromium (Cr) Toxicity: What Are the Physiologic Effects of Chromium Exposure? | ATSDR - Environmental Medicine & Environmental Health Education - CSEM", 2016). Basically, several different Cr forms can be distinguished depending on their valence state, namely Cr(III) and Cr(VI) ("chromium, toxicology", 2016). In fact, Cr(VI) is the main toxicity source due to its higher oxidation and destructive ability (CHROMIUM (III) and (VI), 2015). The source of Cr source is the waste dumps from chromate-producing plants that bring about air and water contamination (Homa, Haile & Washe, 2016).

During exposure to Cr(VI) in the environment, nose and lungs of human will be affected (Henderson, Rebar & Denicola (1979)). Inhalation of Cr(VI) dust is the major factor of respiratory system damage in human and animals (Oliveira, 2012). It also causes asthma, chronic irritation, chronic rhinitis, congestion and hyperaemia (Lindberg & Hedenstierna, 1983).

Besides, Cr(VI) is widely acknowledged as the most common skin sensitizing agent (Cervantes, 2001). The solubility of Cr(VI) would allow the compound to penetrate into our skin and cause painless erosion and allergic responses in human (Fleeger & Deng, 1990). The signs and symptoms of Cr(VI) allergic responses include dryness, erythema, scaling, vesicles, swelling ("Chromium (Cr) Toxicity: What Are the Physiologic Effects of Chromium Exposure? | ATSDR - Environmental Medicine & Environmental Health Education - CSEM", 2016)

2.3 ALUMINIUM TOXICITY

Aluminum is a trivalent cation found in its ionic structure in many parts of animals and plant tissues as well as environment (Jiang et al., 2008). It is the third most pervasive component and the most copious metal in the earth's crust, contribute to roughly 8% of aggregate mineral components (Verstraeten, Aimo & Oteiza, 2008). Due to its reactivity, Al in nature is found in the form of compound with other element.

Incorporation of Al may cause hindrance of protein synthesis and enzyme activity, adjustments nucleic acid capability and changes in the permeability of cell membrane. Toxicity of Al may directly affects hematopoiesis and causes anaemia through diminished heme synthesis, diminished globulin synthesis, and prolonged hemolysis. Aluminum may directly affect the absorption of iron in digestion system (Exley, Swarbrick, Gherardi & Authier, 2009).

2.4 BIOSORPTION

Nowadays, clean technology has become a significant development in the context of environmental biotechnology. Clean technologies are involved in treatment and degradation of waste contamination naturally (Kulshreshtha, Mathur & Bhatnagar, 2014). In the category of clean technologies, biosorption has been developed as an optional innovation which eventually turn into a promising innovation due to its potential application in protection of environment. Biosorption has become a popular clean technology compared to conventional treatment in the context of environmental biotechnology due to its high removal ability, low cost requirement in preparation, minimization of compound and natural ooze, no extra supplement prerequisite, recovery of biosorbent and probability of metal recuperation (S. R. & Saral, 2014). Based on the article reviewed, the microorganisms have a significant ability to accumulate high concentration of metals. The category of microorganisms including bacteria, fungi and algae are basically used as a biosorbent that display a high ability in sorption of heavy metal on their surface.

Yet, the best choice for sorption of heavy metal are algae and fungi because the bacteria are less impervious to heavy metal toxicity. The algae as an autotroph and it require a steady supply of carbon source, oxygen. Moreover, because of little size in bacteria and algae, both of them are hard to be gathered and require laborious solid liquid separations. Therefore, fungi has become more qualified as an adsorbent for heavy metal expulsion than some other microbial mass due to their extraordinary resistance towards poisonous metals and other unsuitable ecological conditions and it contain an ability to grow continuously under different natural conditions (Kohlmeier et al., 2005). It has been shown that numerous fungal species display high biosorptive possibilities (Wang & Hou, 2010; Li, Liu, Fan, Ai & Shan, 2011). Some of the chemical compounds are trapped by biosorption via ion exchange processes, covalent binding interaction and adsorption (Boamah et al., 2015). Hence, the polar charge in the proteins, lipids, amino acids and structural polysaccharides such as chitin might be included in biosorption process (Kulshreshtha, Mathur & Bhatnagar, 2014). In this case, mushrooms can be utilised to decrease the level of ecological contamination by biosorption of nickel, chromium, copper and cadmium (Kamarudzaman, Chay, Amir & Talib, 2015).