A NOVEL WHOLE CELL BIOSENSOR INCORPORATING *Haematococcus pluvialis* FOR PESTICIDE DETECTION IN WATER

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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

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ACKNOWLEDGEMENT

I would like to take this golden opportunity to show God my sincere gratitude in guiding me through my degree years. I want to express my deepest gratitude towards everyone who has been present and helped me in one way or another. I want to especially thank my supervisor Dr. Ling Shing Wong who has supported me patiently, helped me to improve my scientific writing skills and provided me aspiration and motivation to go forward and to go for big. Thank you Dr. Wan Hee Cheng for having shown great kindness in helping me in the laboratory. A special thank you to my parents and my little brother for their continuous support and love throughout the years. I want to thanks Dr. Geeta Selvarajah and Ms. Emily Quek who greatly helped me in my thesis writing through their workshop done on referencing. Thanks to Dr. Ghim Hock Ong, who with the collaboration of other lecturers organized a workshop for assisting students with performing statistical analysis. Lastly, I would also like to thank my laboratory colleagues Suzan Nakayiza, Albert Ong, Nur Diyana and Fan Wei Jiong who helped me build my team spirit and even more.

ABSTRACT

There are major environmental and health concerns associated with pesticide presence in water. Conventional detection methods are expensive and laboratory based inadequate for *in situ* and real time monitoring of the pollutants. As such this research was focused on integrating *Haematococcus pluvialis* in a novel whole cell biosensor using *de novo* astaxanthin production as bioreporter for the detection of the pesticides atrazine, diuron and methyl parathion in water. The microalga was cultured over 29 days and the growth pattern was successfully determined. The chlorophyll and astaxanthin cell contents were also determined spectrophotometrically. Immobilization of the cells was done in agarose gel and the response of the cells was measured in terms of percentage change in astaxanthin level by recording OD readings from a spectrophotometer with wavelength set at 482 nm before and after the exposure of the cells to the pesticide analyte. Optimum response of the biosensor towards the pesticides was 15 minutes after the exposure using day-3 cell culture at 2.50 x 10^5 cells/cuvette as cell density. The biosensor was exposed to different 0.0010 mg/L, 0.0025 mg/L, 0.0050 mg/L, 0.0100 concentrations (mg/L, 0.1000 mg/L, 1.0000 mg/L, 5.0000 mg/L and 10.0000 mg/L) of each of the pesticides at the optimized pH 7.0. The biosensor showed high sensitivity to the range of concentration of the three pesticides used in this experiment with highest response recorded at pesticide concentration lower than 0.0010 mg/L. Therefore, the biosensor can be used as a screening system for environmental samples to indicate the presence of pesticides ad at the same time serves as a water safety warning system.

DECLARATION

I hereby declare that the work in this proposal is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Dr. Wong Ling Shing.

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08/05/2017

Dr. LING SHING WONG

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LIST OF ABBREVIATIONS

°C	degrees Celsius
µl	Microlitre
$CaCl_2.2H_2O$	Calcium Chloride Dihydrate
$Co(NO_3)_2.6H_2O$	Cobalt(II) Nitrate Hexahydrate
Cu	Copper
$CuSO_4.5H_2O$	Copper(II) Sulfate Pentahydrate
EDTA	Ethylenediamine-N,N,N ,N -tetraacetic Acid
EPA	Environmental Protection Agency
$FeSO_4.7H_2O$	Iron(II) Sulfate Heptahydrate
FDA	Food And Drug Administration
h	Hour
H_3BO_3	Boric Acid
H. pluvialis	*Haematococcus pluvialis*
H_2SO_4	Sulfuric Acid
K_2HPO_4	Dipotassium Hydrogen Phosphate
KH_2PO_4	Monopotassium Phosphate
KOH	Potassium Hydroxide
IUPAC	International Union of Pure and Applied Chemistry
L	Litre
mg/L	Milligrams per litre
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$MnCl_2.4H_2O$	Manganese(II) Chloride Tetrahydrate
min	Minutes
mL	Millilitre
MoO_3	Molybdenum(VI) Oxide
NaCl	Sodium Chloride
$NaNO_3$	Sodium Nitrate

nm	Nanometre
OD	Optical density
Pb	Lead
psi	Pound per square inch
ROS	Reactive oxygen species
rpm	Revolutions per minute
t	Time
Zn	Zinc
$ZnSO_4.7H_2O$	Zinc Sulfate Heptahydrate

CHAPTER 1

INTRODUCTION

Pesticides are chemical or biological substances use for killing or slowing growth of pests such as insects and weeds which hinders the growth of crops (Bhadekar, Pote, Tale, & Nirichan, 2011). Their application is primordial in the agricultural industries since one third of all agro products worldwide are produced using pesticides (Zhang, Jiang, & Ou, 2011). Pesticide application increase crop productivity, lowering labor cost and protecting crops from pathogens and vector borne diseases (Bhadekar et al., 2011).

These chemicals can move from treated agricultural areas to non-target regions. This leads to problems like less efficient pest control and environment contamination (Tiryaki & Temur, 2010). To attain sufficient leaf coverage to prevent pest attack farmers use pesticides excessively and cause them economic loss (Ben-Zur, Hake, Hassoon, Bulatov, & Schechter, 2011). Water pollution happens when pesticides from agricultural fields end up in rivers, lakes, ground water or sea through leaching, run offs or atmospheric depositions. Ingestion or dermal contacts with such water are routes how human get exposed to the pollutants (Briggs, 2003). The acute and chronic diseases that might arise include kidney failure, nervous system damage, cancer, diarrhea, vomiting and gastroenteritis (Mashhood & Arsalan, 2011). These pesticides have detrimental effect on the environment as they can bioaccumulate in the food chain (DeLorenzo, Scott, & Ross, 2001b). Other consequences include development of resistant pests, intoxication of aquatic animals like the endangered river dolphin (Aktar, Sengupta, & Chowdhury, 2009). Thus, it is important to detect their presence in water.

Conventional ways of pesticide detection comprise of high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), X-ray diffraction and nuclear magnetic resonance (NMR).They have high sensitivity and reliability (Bhadekar et al., 2011). Nevertheless, their common limitations include complexity of operation thereby requiring highly skilled technician. Extensive pretreatment of samples needed is time consuming and unsuitable for *in situ* detection (Kumar, Upadhay, Wasit, Singh, & Kaur, 2013; Ben-Zur et al., 2011).

Biosensors are deemed alternatives because of their high sensitivity, inexpensive instrumentation, room for instrument miniaturization and portability. The instrument is composed of a biological recognition element coupled with a transducer (Bhadekar et al., 2011). The signal transduction is made through optical, mass-based and electrochemical means used singly or in combinations. Examples of bioreceptor used include nucleic acids, enzymes, antibodies, microorganisms and whole cells (Buonasera, Pezzotti, Pezzotti, Cano, & Giardi, 2015). Whole cells like cyanobacteria, yeast, plant cells, fungi and algae cells are preferred because they are bioindicators which respond when the biosensor is exposed to toxicants (Teo & Wong, 2014). Microalgae have been used as they are easily cultured, immobilized and reproduce fast. They can detect the presence of pollutants because upon their intake, the pollutants interfere in their enzymatic reactions and effect in the photosynthetic electron transport pathways (Buonasera, Lambreva, Rea, Touloupakis, & Giardi, 2011). These changes can be detected via spectrophotometry, colorimetry, bioluminescence, fluorometry, optical or conductometry transducer (Sanchez-Ferandin, Leroy, Bouget, & Joux, 2013; Durrieu et al., 2006).

Many other algae species have been reported suitable for detecting pollutants but none studied the microalgae *Haematococcus pluvialis*is. This microorganism is the best for naturally producing astaxanthin (Shah, Liang, Cheng, & Daroch, 2016). *Heamatococcus pluvialis* accumulates astaxanthin under stress conditions notably nitrogen or phosphate deprivation, high/low temperature, increased salinity and combinations of these conditions (Collins et al., 2011; Tripathi, Sarada, & Ravishankar, 2002). In this research, the *in vivo* astaxanthin response of the cells will be noted using a spectrophotometer in the view of using astaxanthin as a bioreporter for the detection of the pesticides atrazine, diuron and methyl parathion.

The aims of this study were:

1. To determine *H. pluvialis* growth pattern.
2. To determine the conditions for optimum response of the algae cells.
3. To determine the response of the biosensor to atrazine, diuron and methyl parathion.

CHAPTER 2

LITTERATURE REVIEW

2.1 PESTICIDES

Pesticides are highly needed in agriculture since crop loss is estimated to be 14% caused by insects, 13% by plant pathogens and another 13% as a result of weeds. Crop loss in turn means economic loss to planters (Aktar et al., 2009). This is the reason why nearly one third of all agricultural products are results of pesticides application. It is thus undeniable that pesticide application result into higher crop yield and better food produced (Damalas & Eleftherohorinos, 2011; Hellar & Kishimba, 2005). It is estimated that crop lost due to pest will be 78% for fruits, 54 % for vegetables and 32% for cereals without pesticide application (Zhang et al., 2011). The top worldwide pesticide utilizer is Europe seconded by Asia. The top pesticide manufacturers/utilizers are France, the United States of America, Japan, Brazil and China (Zhang et al., 2011). Most vector borne diseases are controlled by killing the vectors thus pesticides help to keep disease outbreaks like malaria under control (Aktar et al., 2009).

Synthetic pesticides are commonly classified into three groups; based on the mode of action, the composition or the target pest (Zacharia, 2011). Based on mode of action, the pesticide can bring its effects in a systemic or non-systemic way. The former is when the pesticides penetrate plant tissues and is circulated round the plant through the vascular tissues for effecting. The latter effect on insects only upon contact without requiring transport throughout the pests. Systemic pesticides include glyphosate and 2, 4-D (Zacharia, 2011). Classification based on target pests is where the pesticides are named after the pests they act against, for instance insecticides and herbicides act on insects and weeds respectively (Zacharia, 2011). Pesticides categorized based on their chemical composition are subdivided into four main groups notably; carbamates, organochlorines,