THE POTENTIAL APPLICATION OF *CYANOBACTERIA (A. CYLINDRICA)* AS WHOLE-CELL BIOSENSOR TO DETECT PESTICIDES

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

FACULTY OF SCIENCE, TECHNOLOGY,
ENGINEERING AND MATHEMATICS
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA

2015

TABLE OF CONTENT

ABSTRACT

Environment is often polluted with more than one type of toxicants such as pesticides and *Anabaena cylindrica* had the potential to be applied in a whole-cell biosensor to detect pesticides. *A. cylindrica* had been immobilized in 1% agarose gel and exposed to five different concentrations of three pesticides which were 2,4-dichlorophenoxyacetic acid (2,4-D), methyl parathion and atrazine. The response were determined by measuring the absorbance reading at 450 nm, which was the optimum absorption wavelength of β-carotene. Based on single toxicity test, there was an increase in the percentage of absorbance change for all three pesticides due to the increased production of β-carotene to overcome the oxidative damage caused by pesticides. As for combined toxicity test, there was an in the percentage of absorbance for all combination of pesticides. All combination of pesticides at 0.01 mg/L showed a synergistic effect when compared to single toxicity testing and this was due to pesticides were unable to interact with one another at low concentration to form less toxic complex. At concentration higher than 0.01 mg/L, antagonistic effect was observed for all combination of pesticides as the high concentration enable them to interact with one another to form less toxic complex. However for methyl parathion and atrazine in combination, which produced a synergistic response at 0.10 mg/L as well.

ACKNOWLEDGEMENT

I would like to thank all those who helped me a lot in this project, especially a special thanks to Dr Wong Ling Shing for being a very helpful supervisor to guide me throughout this project and thesis writing. I would also like to thank other supervisors and lecturers who gave me advices in this project and thesis writing as well as thank to my fellow course mates, Saktis Paul and Tan Yeong Hwang for helping me out in this research project. Finally, I would like to thank my family for supporting me a lot in this research project besides encouraging and motivating me to go on.

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LIST OF FIGURES

LIST OF ABBREVIATIONS

HPLC	high performance liquid chromatography
NMR	nuclear magnetic resonance
°C	degree Celcius
mL	milliliter
rpm	round-per-minute
µL	microliter
LAF	laminar air flow
nm	nanometer
g	gram
OD	optical density
ppm	part per million
2,4-D	2,4-dichlorophenoxyacetic acid
t	time
OD	optical density
mg	milligram
L	liter
w/v	weight/volume percentage

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Pollution is a common problem around the globe which includes land, air, water, and sound pollution. Pollution is mainly caused by human activities is the agricultural, industrial, domestic and commercial sector (Mondal, 2014). These pollutants have negative impacts on human health such as causing skin and respiratory diseases as well as disrupt the balance of ecosystem. Environmental pollutants affect majority of the biosphere (Lone et al., 2008). The sources of such pollutants can be from industrial wastewater, human sewage, excessive disposal and littering of waste and so on (Sen, 2014). The accumulation of toxicants affect not only the land, but may enter the ground and pollute groundwater as well as other water bodies such lakes and rivers (Sen, 2014). Hence, the detection of such toxicants in the environment is crucial.

There are several methods for detecting environmental toxicants. The methods include physical, chemical and biological methods. Physical methods include monitoring pH and dissolved oxygen, and colorimetry. Chemical methods include high performance liquid chromatography (HPLC), spectrophotometry and nuclear magnetic resonance (NMR) (Nair & Kuriakose, 1999). As for biological method, the commonly used method is using an indicator species. All these methods are either time-consuming or costly or both (Chatham & Blackband, 2001). Thus, scientists began to come out with a cheaper and better alternative which is the biosensor.

Biosensor works based on the basis of signal transduction (AZoSensors, 2013). It is composed of a biological element that responds to changes caused by specific toxicant and a transducer that measures such changes (Mandal, 2014). Biosensor has several advantages other than cost-effective if compared to other detection methods. One of the advantages is that it allows fast and continuous measurement of the toxicants (AZoSensors, 2013). It also has high sensitivity and specificity towards a specific substance (Sadana, 2003). Besides that, it requires less usage of chemicals and reagents for calibration which saves up a lot of cost as well as has fast response time and more convenient (AZoSensors, 2013).

The biosensor that is going to be used in this project is whole-cell biosensor. This is because it shows good linearity and high sensitivity in detecting contaminants and toxicants (Yagi, 2006). The whole cell biosensor also has higher stability compared to protein-based or immunoassay-based biosensor (Yagi, 2006). Besides that, the detection using whole-cell biosensor can be easily quantified as the amount of protein produced in order to counter the effect of toxicants can be easily assayed using spectrophotometer, thus allowing further quantification (Yagi, 2006).

In this project, a cyanobacteria's behavior towards both single and different combinations of toxicants will be tested in order to allow better design of biosensor. The objectives of this project are:

1. To determine the growth pattern of *Anabaena cylindrica*
2. To determine the optimum absorbance wavelength for β-carotene
3. Optimization of day of culture and cell concentration of *A. cylindrica* and exposure time
4. To study the response of *A. cylindrica* to different concentrations and combinations of pesticides

CHAPTER 2

LITERATURE REVIEW

2.1 HUMAN ACTIVITIES AND TOXICANTS

Human activities had been releasing a huge amount of toxicants such as heavy metals and pesticides into the environment (Wong et al., 2013). These toxicants mainly pose a potential risk to both human and the environment (Palomares et al., 2009) and these toxicants usually interact with one another to form new toxicants or produce various effects (Folt et al., 1999). However, the effects of these toxicants have been tested individually, but in the environment the organisms living there are exposed to a different combination of the toxicants. For instance, a living microorganism that can survive and counter the effect of a particular toxicant may not survive if the particular toxicant is mixed with other types of toxicant. In another way round, a living microorganism that cannot survive in the presence of a particular toxicant may survive when the particular toxicant is present together with other toxicant. When these toxicants accumulate in the aquatic system, it will disrupt the balance of the aquatic system by reducing organism abundance (Fleeger et al., 2003). When this happens, marine products that are being consumed by humans might contain those toxicants. These toxicants also cause disease, disability, and early death among humans (Wayman, 2013). A growing body of research also suggested that the exposure to these toxicants posed a risk to women's health as well as child's health and development (Harrison et al., 2009). Furthermore, these environmental toxicants had been implicated with cancer among the human population.

2.2 TOXICANTS AND THEIR EFFECTS

2.2.1 2,4-dichlorophenoxyacetic acid (2,4-D)

2,4-D is a colorless and odourless substance that is used as herbicide to control the spreading of broad-leaf weeds in the agricultural field (EPA, 2013). Often, this herbicide can be found at polluted water sites due to its high usage in the agricultural sector and the resultant flow from agricultural farm to river or water sources. The maximum contaminant level goal as set by EPA for 2,4-D was 0.07 mg/L and this was made as standard in order to prevent any potential health problem if people happened to drink the contaminated water. If the concentration of 2,4-D in water exceeded the standard concentration, it will have a deleterious effect on human health. Most of the noticeable effects are mostly problems associated with kidney, liver and adrenal glands.

2.2.2 Atrazine

Atrazine is also one of the most commonly used pesticides to control grassy weed problem (EPA, 2013) and it very toxic to human as well (ATSDR, 2003). Due to its high toxicity, its usage had been restricted since 1993 by the EPA agency. The maximum contaminant level goal set for atrazine by EPA is 0.003 mg/L and if any concentration of atrazine is found to exceed this standard, it will lead to serious health problem. Not only is atrazine toxic at a very low concentrations, but it is hard to be detected and removed using current available analytical methods. Most water sources were found to be contaminated with atrazine with concentration exceeding 0.003 mg/L, thus making the detection possible. Atrazine can disrupt the hormonal activity in human even in a very low concentrations. Some of the effects include miscarriage, reduced male fertility, low birth weight and so on. Besides, recent research also found out the high carcinogenic potential of atrazine where the capability and possibility of atrazine to cause cancer are to be studied further in detailed.

2.2.3 Methyl Parathion

Methyl parathion is a man-made organophosphate pesticide that is commonly used to control insect pests (EPA, 2000) and it is one of the most toxic pesticide (EPA, 1999).This pesticide was not only toxic to insect, but poses potential acute toxicity to human as well especially children. This pesticide can be consumed by human not only from water sources, but through diet as well. Studies have revealed that this pesticide had found to pose unacceptable dietary risk to children through consumption, suggesting the the active ingredient of methyl parathion can still remain inside the crop even after harvesting and washing. A short term exposure to methyl parathion may affect nervous system function. Since methyl parathion inhibits cholinesterase, an enzyme that is responsible for breaking down acetylcholine to help in signal transmission, thus more acetylcholine will accumulate and lead to improper functioning of the nervous system. Despite its high toxicity, methyl parathion is still being used on several major crops but efforts to reduce its usage had been focused on in order to reduce damage to both the environment and living organisms affected by it.

2.3 CYANOBACTERIA

Cyanobacteria are aquatic, photosynthetic microorganisms that are capable of carrying oxygenic photosynthesis. They were considered the oldest group photosynthetic microorganism and also the first photosynthetic organism to carry oxygen-dependent photosynthesis (Wong et al., 2014). They appeared as blue-green in color due to presence of phycocyanins and they have chlorophylls to carry out photosynthesis. Besides chlorophylls, they also have other accessory pigments such carotenoids and phycobilins (Wong et al., 2014). Although similar to eukaryotic algae in terms of photosynthesis capability and appearance, they differ in that they are prokaryotes (Vincent, 2009). They lack of internal organelles, do not have true nucleus. Considered as a bacteria, they have a peptidoglycan layer in their cell wall similar to that found in bacteria. However, they could be easily distinguished from bacteria due to presence of photosynthetic apparatus and oxygen production, which were absent in bacteria.

Cyanobacteria are most commonly found in aquatic environment such as lakes, rivers, wetlands and so on (Vincent, 2009). They played a major role in regulating carbon, nitrogen and oxygen balance of many aquatic environment. There were evidences suggesting that cyanobacteria were derived from an endosymbiosis where a cyanoabcterium was engulfed by a colorless eukaryotic cell and they were responsible for the accumulation of oxygen in the Earth's atmosphere during ancient times.

In an aquatic environment with presence of sunlight, cyanobacteria can photosynthesize using water as an electro donor and in turn, produces oxygen. They are very resistant to UV radiation and reactive-oxygen species (ROS) due to production and presence of various carotenoids such as β-carotene, canthaxanthin, echinenone and so on. Those carotenoids are anti-oxidant that can effectively scavenge the ROS and other oxidative agent capable of causing oxidative stress and damage (Vincent, 2009).

2.3.1 *A. cylindrica*

A. cylindrica is a type of filamentous cyanobacteria which are capable of carrying out nitrogen-fixing (Walloon, 2010). They grow in long filaments of vegetative cells with heterocyst form in between irregularly. The photosynthetic cells appear as green while heterocyst appears as colorless while in some cases, light green or yellow. The photosynthetic cells carry out photosynthesis while heterocyst carries out nitrogen fixation and both processes have to be carried out separately due to inactivation of a nitrogen-fixing enzyme, nitrogenase caused by presence of oxygen (SERC, 2007).

A. cylindrica are commonly found at polluted aquatic sites and are photosynthetic prokaryotes that can harvest light energy using chlorophyll a and other accessory pigments such as carotenoids (Vincent, 2009). This type of microbes are commonly found in water sources such as lakes, ponds, springs, rivers where toxicants mostly accumulate, thus making them a suitable subject to be studied for the development of biosensors. Besides that, cyanobacteria is preferred in the application of biosensor is because they are cheaper to culture than higher organisms (Shao et al., 2002). In addtion, they can also respond rapidly to toxicants as well as indicating the bioavailability of a particular toxicant in which cannot be done by chemical analysis. The figure of *A. cylindrica* is shown in figure below:

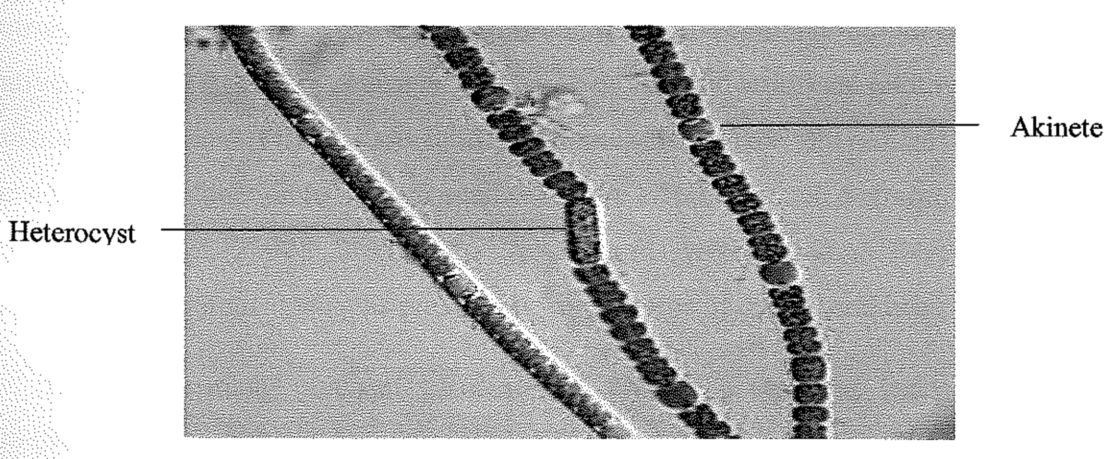

Figure 2.1 The structure of *A. cylindrica*