WHOLE CELL BIOINDICATOR USING *Anabaena cylindrica* AND CAROTENOIDS AS A REPORTER

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ABSTRACT

Pollutants like heavy metals have detrimental effect on humans and other organisms, therefore a fast and easy detection method is required. For monitoring purposes, whole cell biosensors are good alternatives as they are portable, allow rapid detection and generate reliable results. The main objective of this experiment is to study the effects of single and combined toxicity response of *Anabaena cylindrica* towards copper (Cu), cadmium (Cd) and lead (Pb). Through standardised experimental parameter, *A.cylindrica* of day 7 culture cells with cell density = 0.5 A were immobilised in 1% agarose and exposed to various concentrations (0.001 mg/L, 0.010 mg/L, 0.100 mg/L, 1.000 mg/L and 10.000 mg/L) of heavy metals. The effect of heavy metal on carotenoids, produced during photo-oxidative stress was measured using OD_{450nm}. The results showed predominantly antagonistic effect of combined toxicity of different combination of heavy metals. The response of *A.cylindrica* to heavy metals enables the build-up of biosensors capable of quantitative and qualitative analysis of single and combined heavy metals' toxicity. Thus, a whole cell bio indicator was successfully constructed using carotenoid as a reporter.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	degrees Celsius
IUPAC	International Union of Pure and Applied Chemistry
DNA	deoxyribonucleic acid
Cr	chromium
As	arsenic
Cd	cadmium
Pb	lead
Cu	copper
ROS	reactive oxygen species
PVC	Polyvinyl chloride
Cu^{2+}	copper ion
Cr^{2+}	chromium ion
Cd^{2+}	cadmium ion
Zn^{2+}	zinc ion
Hg^{2+}	mercury ion
Pb^{2+}	lead ion
mg/L	milligram per liter
µl	microliter
µl	microlitre
OD	optical density
$CuSO_4$	lead(ii) sulphate
$Cd(NO_3).4H_2O$	Cadmium nitrate tetrahydrate

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Heavy metals are abundant in the Earth's crust. They are also a common by-product of industrial and agricultural activity (Turdean, 2011). Heavy metals are metals with atomic mass within the range of 63.5 g mol^{-1} to 200.6 g mol^{-1} in the periodic table. For animals, some heavy metals are required in trace amounts for growth (Turdean, 2011). Heavy metals that flow downstream from industrial plants can bind to the surface of microorganisms, where they accumulate within their cells and become toxic. Heavy metal accumulation in excess amount could be detrimental to humans and other organisms (United States Department of Agriculture, 2000). Most commonly known problem-causing heavy metals are cationic metals such as mercury, cadmium, lead, zinc and copper; however there are still anionic compounds like molybdenum, selenium and boron which can also cause toxic effects and are classified as toxicants (United States Department of Agriculture, 2000). This creates the need for analytical devices capable of detecting those heavy metals and other toxicants in the environment.

Existing analytical tools like atomic absorption and emission spectroscopy, inductively coupled plasma spectroscopy and high-performance chromatography require sophisticated techniques that limit them to only laboratory uses by skilled personnels and takes longer time to analyse a sample (Mozaz et al., 2006). Thus, there is a need for rapid, low-cost and an on-site monitoring device with the ability to measure complex matrices with minimal samples. This leads to the development of biosensor, as it had demonstrated great potential as an analytical tool for environmental monitoring (Mozaz et al., 2006). Biosensors, according to IUPAC nomenclature is a device that measure the biochemical reaction mediated by isolated enzymes, immune-systems, tissues or whole cells using a transducer (Mozaz et al., 2006). Biosensor could be divided based on the types of transducer (detection mode) used, like optical, electrical electrochemical and mass sensitive thermal or types of

analyte like enzymes, proteins, antibodies, DNA, organelles, microbial cells and plant or animal tissue (Monošíka, Streďanskýb & Šturdík, 2012; Corcuera & Cavalieri, 2003).

A whole cell biosensor showcases the bioavailability of toxicants like heavy metals to biological systems, unlike conventional analytical tools already in market (Verma, 2011). Both quantitative and qualitative analysis is possible through the measurement of biochemical responses of living cells towards pollutants such as heavy metals and pesticides. In a whole cell biosensor, the transducer detects and converts those biochemical responses into digital outputs (Wong, 2014).

Carotenoids are non-nitrogenous molecules responsible for the yellow, orange or red pigments of biochromes (carotenes and xanthophylls), that is the reason why some leaves appear red or yellow during autumn (University of California, 2006). They are accessory pigments of photosynthesis; they do not participate directly in trapping light energy (University of California, 2006). In cyanobacteria, carotenoids do not just play a role in the photosystem, but also protects them from photo-oxidative damages (Liang, 2006). Cyanobacteria's growth conditions and environmental factors such as presence of high concentration of heavy metal affect biosynthesis and causes accumulation of carotenoids (Hirschberg, 1994). Carotenoids absorb wavelength best within the range of 400 nm to 480 nm according to Wong (2014). Thus using spectrophotometer analysis, a simple whole cell biosensor using cyanobacteria can be constructed, making the carotenoids as the reporter species.

In this research, the main objective is to study the optical response of carotenoids towards single and combined heavy metals toxicity, which is the theoretical study of a biosensor. This objective can be achieved through:

i. The determination of cell growth of *A.cylindrica,* a kind of cyanobacteria which is rich in carotenoids.
ii. Standardisation of experimental conditions such as day of cell culture used for immobilisation, exposure time and cell density.
iii. The immobilisation of *A.cylindrica* cells in the cuvettes.
iv. The determination of response of the cells towards the exposure of single and combined toxicity of heavy metals.

CHAPTER 2

LITERATURE REVIEW

2.1 CYANOBACTERIA

They are aquatic and photosynthetic bacteria. They lack the membrane bound organelles like nucleus and chloroplast, which is much similar to bacteria (Soil & Water Conservation Society of Metro Halifax, 2007). They are commonly known as blue-green algae, yet they are not true algae as they do not reflect any relationship to algae (University of California, 2006; Speer, 1995).

Cyanobacteria are highly versatile and are able to flourish anywhere with the presence of sunlight (Steur, Knoop & Machne, 2012). They are aquatic and photosynthetic bacteria that can synthesise their own food. They are unicellular organisms that grow in colonies or filaments; they are small but larger than other bacteria. They lack flagella but are able to move due to swaying motion of its filaments. Most cyanobacteria possess nitrogen fixing properties and are capable of converting inert atmospheric nitrogen into nitrates or ammonia, which are used by plants (Speer,1995).

There are three basic pigments in cyanobacteria; chlorophyll, carotenoid and phycobilins. Chlorophyll consists of a porpyrin ring which easily gains or loses electron, thus is the fundamental to photosynthesis. It provides energised electrons to other molecules. There are three types of chlorophyll; chlorophyll a, found in all plants, algae and cyanobacteria, chlorophyll b found only in green algae and plants, and chlorophyll c found only in photosynthetic chromista and dinoflagellates. Next are the carotenoids, an accessory pigment, as they only transfer absorbed sunlight energy to chlorophyll. Lastly phycobilins, only found in cyanobacteria and rhodophyta, dispersed throughout the cytoplasm. This pigment fluoresce at a particular wavelength (Speer,1997).

2.1.1 *Anabeana cylindrica*

A. cylindrica is a cyanobacterium, member of the *Nostocaceae* family. It is a filamentous structure. The cells are cylindrical and the trichomes have a consistent width of 4 μm with no branching (Protist Information Server, 2013). Under unfavourable conditions, *A. cylindrica* forms heterocysts or akinetes, shown in Figure 2.1 (a) and (b), to adapt to its environment. The nitrogen fixation is carried out by heterocysts, specialised cells which are larger and have a thick cell wall (Crane, 2010). These specialized cells provide an anaerobic environment for the enzyme nitrogenase, which fixes atmospheric nitrogen into ammonium (Vincent, 2009). Akinetes are climate-resistant spores, with thick cell walls that are resistant to drying and freezing. These cells can remain dormant for long periods and germinate only when the environmental conditions are favourable (Crane, 2010).

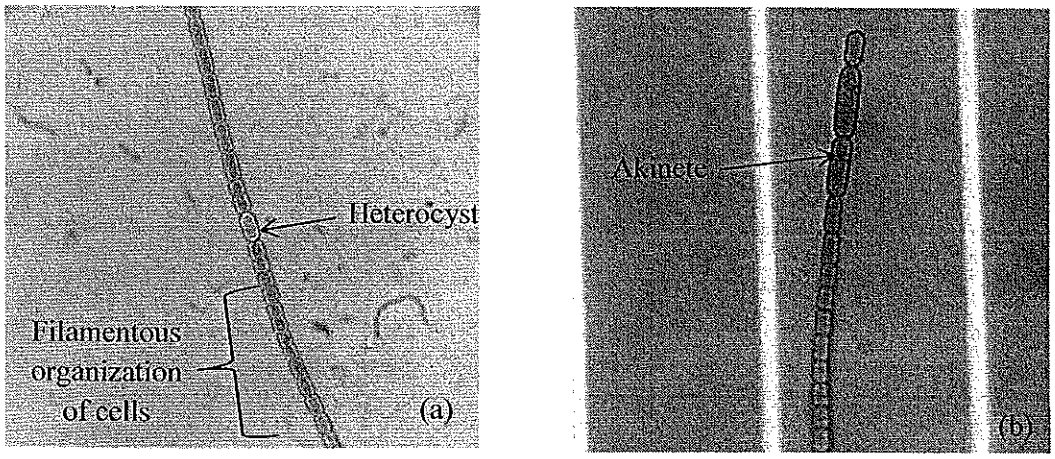

Figure 2.1 (a) and (b) Structure of *A. cylindrica* and the presence of both heterocyst and akinetes observed at 400x magnification under light microscope.

2.2 CAROTENOIDS

All photoautotroph have carotenoids, an isoprenoid pigments that are essential part of photosynthesis (Cunningham, Lee & Gantt, 2006) and protection against photo-oxidative damages. It is found omnipresent in cyanobacteria. Carotenoids help in light harvesting and act as an antioxidant. The types of carotenoids produced in cyanobacteria are *β*-carotene, echinenone and myxol glycoside (Takaichi, Maoka, Takasaki & Hanada, 2009). Zeaxanthin (3,3′-dihydroxy-β-carotene), a product of

hydroxylation of β-ring on β-carotene acts as photo-protective pigment in cyanobacteria (Cunningham, Lee & Gantt, 2006). Photo-oxidative stress is often due to absorption of excess excitation energy, sunlight energy (in excess) which leads to harmful reactive oxygen species (ROS) in cyanobacteria (Reddy & Raghavendra, 2006).

2.3 IMMOBILISATION OF *A.cylindrica*

Immobilization of cells increases the stability of the cells, reduces the risk of contamination, keeps the cells closer to the transducer, therefore increasing the efficiency in receiving signals from reporter (Teo, 2014).There are 4 different ways for coupling the biological and sensor elements; membrane entrapment, physical adsorption, matrix entrapment, and covalent bonding (Mohanty & Kougianos, 2006).

Membrane entrapment technique was used in this experiment. Membrane entrapment is caging of an enzyme or a whole cell through covalent or non-covalent bonds within a gel or fibre structure. It prevents leakage and increased mechanical stability. Membrane entrapment is achieved through polymerization of polymer matrix and the enzyme, thus forming small bead or a film on a solid support. In this experiment, the whole cells are immobilised in agarose (gel) (Datta, Christena & Rajaram, 2012).

2.4 BIOSENSORS

Biosensors are promising analytical tools applicable in clinical diagnosis, food industry, environment monitoring and in other fields, where rapid and reliable analysis are needed (Monošík, 2012).

Biosensors are low-cost and highly efficient devices which consist of the combination of a highly specific biological element coupled to a transducer (physical element). This specific biological element recognises the analyte and creates a certain biochemical response which is detected by the transducer as shown in Figure 2.2 below. These bio-elements or bio-receptors can be enzymes, antibodies, living cells or tissues, while the physical element could be electrical, thermal or optical signals.