THE RESPONSE OF *Anabaena cylindrica* TO SINGLE AND COMBINED TOXICITY OF NICKEL, ALUMINUM AND LITHIUM

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Abstract

Rapid development of human activities leads to serious metal pollution. In the real polluted environment, the metals usually do not present in single pure form, but occur in mixtures in the environment. Thus, there is a need to study the response of whole cell towards combination of metals in order to develop a whole cell biosensor to detect metal pollutants in environmental samples. The objective of this study was to determine the response of immobilized *Anabaena cylindrica* toward single and combination toxicity of nickel (Ni), aluminum (Al) and lithium (Li). Three cell concentrations (OD_{700nm}= 0.5 A, 1.0 A and 1.5 A) of *A. cylindrica* from Day 5, 6 and 7 cultures were made and then immobilized with 1% agarose. The immobilized cell were exposed to 0.100 mg/L Ni and OD_{400nm} was measured after 1 hour, 2 hours, 6 hours and 24 hours exposure. Immobilized *A. cylindrica* taken from Day 5 with OD_{700nm} = 0.5 A gave the highest response after 2 hour exposure and thus used as the optimized condition for the experiment. The immobilized cells showed the increment in change in absorbance at low concentration of single Ni ions. Decrement in change in absorbance was observed at high concentration of single Ni ions. The exposure to single Al and Li ions showed a decrease trend in change in absorbance with increased concentration. The exposure to the combination of metals indicated that synergistic effect was found in the combination of Ni + Al and Ni + Li. The antagonistic effect was found in the combination Al + Li and Ni + Al + Li. High R^2 value for 0.001 mg/L to 0.100 mg/L for single and combined metal exposure indicated that *A. cylindrica* has the potential for biosensor application to detect single and combined metals. High slope values indicated that *A. cylindrica* was sensitive to single and combined metals.

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LIST OF ABBREVIATION

Al	aluminum
ALA-D	δ-aminolaevulinic acid-dehydratase
°C	degree Celsius
g	gram
g/L	gram per liter
Li	lithium
Mg	magnesium
Mn	manganese
mg/L	milligram per liter
mL	milliliter
Ni	nickel
nm	nanometer
OD	optical density
PSI	photosystem I
Pb	lead
rpm	revolutions per minutes (rpm
λ	wavelength

CHAPTER 1

INTRODUCTION

With rapid development of industrialization and urbanization, amount of metal pollutant released into the environment has grown enormously. It becomes an acute problem for many developed country such as China (Wang, Xu, Sun, Liu. & Li, 2013; Xu, Zhao, Zhao, Wang, & Deng, 2014), India (Kaushik et al, 2009) and Malaysia (Sany et al, 2013). Some metals such as nickel (Ni) and copper are micronutrient for living organisms. However, they have the potential risks for health when they are exposed at the high level (Valko, Morris & Cronin, 2005).

Thus, there is a need to develop reliable and fast detection tools to detect metal pollutants. Although conventional analytical methods such as atomic absorption and emission spectroscopy are highly accurate and specific for pollutant detection, they cannot be used outside the laboratory, costly and highly dependent on skilled personnel (Turdean, 2011). Furthermore, these methods are also time-consuming and laborious when screening a large number of samples (Long, Zhu & Shi, 2013). Consequently, the electrochemical method such as biosensors have becoming more and more popular as alternative tools for detection of heavy metal pollution as they are portable, sensitive, less expensive and shorter exposure time (Turdean, 2011; Wong, Lee & Surif 2013b).

A whole cell-based biosensor is a type of biosensor which uses the cells as a reporter that incorporating with the transducer and bioreceptor (Amaro, 2011). It has shown the promising result in some metal pollutants detection (Choe et al, 2012; Wong, Lee & Surif 2013a). Furthermore whole cells are able to reflect the real toxicity and physiological effects of heavy metals to the living organisms (Teo & Wong, 2014).

To date, many studies of whole cell biosensor are focused on the detection of single metal using single type microorganisms (Chouteau, Dzyadevych, Durrieu & Chovelon, 2005; Berezhetskyy et al., 2007; Rahman et al., 2011; Ravikumar, Ganesh, Yoo & Hong, 2012). The metals were detected separately. In the real polluted environment, several types of metal may exist at the same time. To the best of my knowledge, very less biosensor uses the cyanobacteria *Anabaena cylindrica* to detect combined heavy metals. Therefore, there is a potential to develop a whole cell biosensor for detection of combined heavy metals.

In this research, we focused on the usage of cyanobacteria as a bio-component of spectrometry biosensor, designed to detect the presence of heavy metals in environment. The objectives of this project are:

1. Determine the growth curve of *A. cylindrica*
2. Determine the responses of immobilized *A. cylindrica* from different culture day within exponential phase to the metal
3. Determine the responses of different cell concentrations of immobilized *A. cylindrica* to the metal.
4. Determine the responses of immobilized *A. cylindrica* to single and combined exposures of aluminum (Al), nickel (Ni) and lithium (Li).

CHAPTER 2

LITERATURE REVIEW

2.1 ALUMINUM

Al is the third largest abundant element in the earth crust (Akpan, Offiong & Ebene, 2012). It can be found naturally in silicates, bauxite rock and cryolite (NIH Public Access, 2007). Al is an excellent conductor of electricity and heat (Pina & Cervante, 1996) and it is used in the aircraft industries, production of metal alloys, electric industry, and food packaging (World Health Organization, 1998). Al is released into the environment by natural process but recently Al has been found in the serious pollutions due to the industrial activities (Akpan, Offiong & Ebene, 2012; Alahmr et al., 2012). In addition, through the acid rain, Al is released from the natural reservoirs into the water sources (Pina & Cervante, 1996; Sharma, 2003). The solubility of Al is increased in acidic water (Pina & Cervante, 1996; Sharma, 2003; Qaiyum, Shaharudin, Syazwan & Muhaimin, 2011). High concentration of solubilized Al in acidic water caused toxicity to freshwater fish (Poleo et al., 1997; Sharma, 2003). When the birds or other animals consume these freshwater fish, Al is accumulated in their bodies (Sharma, 2003). Accumulation of Al in the human body and presence of high concentration of Al in the drinking water ($\geq$ 0.100 mg/l) were associated with Alzheimer Disease (Gauthier et al. 2000).

Little research was done on detection of Al through whole cell biosensor. Cyanobacteria have potential used for detection of Al as they showed physiological and structural responses to the lower concentration of Al at lower pH (Pettersson, Hallbom & Bergman, 1985; Pettersson, Hallbom & Bergman, 1986). Pettersson, Hallbom, and Bergman (1985) showed that growth of cyanobacteria *Anabaena cylindrica* was significantly reduced when the concentration of Al increased from 0 to 370 μM. Besides that, chlorophyll *a* and phycocyanin concentration in *A. cylindrica* decreased when the Al concentrations increased. The decrease of chlorophyll *a* and phycocyanin has potential use as indicator to detect the presence of Al.

2.2 NICKEL

Ni is a silver-white transition metal (Das, Das & Dhundasi, 2008). It can be found naturally in dusts from volcanic emissions and the weathering of rocks and soils. Ni can be used to make coins, alloys and stainless steels. Ni compounds are used in various industry processes such as electroporation and nickel-cadmium batteries (Duda-Chodak & Baszczyk, 2008). The level of Ni in the environment due to natural process is low but levels can increase when large amount of Ni and their compounds are released from metal refineries and anthropogenic activities (Duda-Chodak & Baszczyk 2008). Ni contamination in water may come from erosion from the earth's crust due to the global climatic change or electroplating industries (Rehman & Shakoori, 2004). The toxicity of Ni to human is depending of its route of exposure and the solubility (Das, Das & Dhundasi, 2008; Duda-Chodak & Baszczyk, 2008). Acute inhalation exposure to Ni can cause dermatitis and Ni compounds are classified as carcinogen by International Agency for Research on Cancer (Das, Das & Dhundasi, 2008).

Lower concentration of Ni could stimulate the growth of algae (Devi Prasad & Devi Prasad, 1981) and cyanobacteria (Shukla et al., 2009; Al-Mousawi, 2010; Chakravartya & Naik, 2014) but the higher concentrations could be potentially toxic (Shukla et al., 2009; Al-Mousawi, 2010; Nohomovich, 2013; Chakravartya & Naik, 2014). Growth of cyanobacteria reduced with increasing Ni concentration (Asthana, Pandey & Singh, 1990; Chris, 2012; Nohomovich, 2013). Chris (2012) showed that Ni treatment with increased concentration reduced chlorophyll pigment in cyanobacteria *Cylindrospermum* sp. Shukla et al. (2009) also indicated that carotenoid and chlorophyll pigment of cyanobacteria *Anabaena doliolum* was reduced when exposed to increased concentration of Ni.

2.3 LITHIUM

Li is the lightest metal and is highly reactive as a pure element therefore it exists naturally as a stable mineral and in compounds. Li has many industrial applications such as production of batteries, synthetic rubbers, alloys, and coolant in nuclear reactors (Kszos & Stewart, 2003). Li is present in the soil and water with low concentration (Aral & Vecchio-Sadus, 2008). However, the Li usage was increased and may cause environmental pollution (Porter & Berno, 2010). Li is a highly mobile element thus the concern about environmental and occupational health related to Li is higher (Aral & Vecchio-Sadus, 2008). Li is not an essential element for life. Metallic Li is classified as health, physiochemical and/or ecotoxicological hazard by the Australia Inventory of Chemical Substances. Metallic Li can cause toxicity to the central nervous system. Li compounds such as lithium chloride and lithium carbonate can lead to death at lower concentration (Aral & Vecchio-Sadus, 2008). Instead of human, Li is also toxic to plant and aquatic organism. High concentration of Li in soil is toxic to the plant and causing chlorosis-like condition (Schrauzer, 2002). High concentration of Li in water resulted in modifying the lipid composition of fish and increase ATPase activity (Tkatcheva, Holopainen, Hyvarinen & Kukkonen, 2007).

Information on effect of Li to the algae and cyanobacteria is lacking. Porter and Bernot (2010) found that increased concentration of Li inhibited or altered microbial activity. Espie, Miller and Canvin (1988) reported that sodium-stimulated photosynthetic oxygen evolution in cyanobacteria was inhibited by Li which acted as competitive inhibitors. The effect of Li to microorganism required further study.

2.4 EFFECT OF METAL TO PHOTOSYNTHESIS

Some trace metals are essential for the plant and phytoplankton. Absence or higher concentration of metal will cause a serious problem to them (Küpper & Kroneck, 2005; Aggarwal et al., 2012). For example, copper is found to associate with plastocyanin, which is an important component of electron transport chains in photosystem I (PSI). Copper deficiency will reduce PSI electron transport and thus affects photosynthesis (Küpper & Kroneck, 2005). High concentration of copper can substitute magnesium in the chlorophyll thus damaging the structure and function of the chlorophyll. Photosynthesis affected by metals indirectly and directly (Aggarwal et al., 2012).

In indirect effect, accumulation of metal (such as Ni and Pb) in leaves caused stomatal malfunction or closure by disturbing thylakoid membranes (Bazzaz, Carlson & Rolfe, 1974). The enzymes δ-aminolaevulinic acid-dehydratase (ALA-D) was sensitive to metals and inhibited by them. ALA-D is important for biosynthesis of chlorophylls thus excess metals led to decrease of chlorophyll production (Gonçalves et al., 2009). Other enzymes involving in the photosynthesis such as water-splitting enzymes and NADPH-oxidoreductase were also found to be sensitive to the metals. Metals bind to sulfhydryl (SH)-groups of these enzymes and lead to inhibition (Aggarwal et al., 2012).

In direct effect, metal bind to various sensitive sites of the photosynthetic apparatus. In chloroplast, metals disturb architecture of thylakoid membranes and change the photosynthesis process (Parmar, Kumari & Sharma, 2013). Excess metals would cause oxidative stress on phototrops. Free metal ions could induce the damage by reducing hydrogen peroxide to highly reactive hydroxyl radical during photosynthesis. Such reactive oxygen species can cause oxidative damage to the lipids and proteins that embedded in the thylakoid membranes (Latifi, Ruiz & Zhang, 2008).

2.5 CYANOBACTERIA

Cyanobacteria are prokaryotic phototrophs. They are widely distributed in lakes, ponds, and rivers. They were the first organism which evolved to produce oxygen on Earth (Vincent, 2010). They have no nuclei and have peptidoglycan cell wall that are similar to gram-negative bacteria. Cyanobacteria have chlorophyll *a* and all of them contain phycobilinproteins which is accessory pigments required for photosynthesis. Phycocyanin which is one class of the phycobilinproteins, giving most of the cyanobacteria cells their appearance as blue-green color (Madigan, Martinko, Stahl & Clark, 2010).

Cyanobacteria can be divided into several groups based on morphology. Unicellular and filamentous forms are known (Madigan et al, 2010). *Anabaena cylindrica* are able to form filamentous body that called trichome. Trichme mainly consists vegetative cells which responsible for photosynthetic growth (Vincent, 2010). When environment conditions are not favored for growth, vegetative cells differentiate into heterocysts and akinetes (Hori et al., 2002; Olli, Kangro & Kabel, 2005; Heng, Lee, & Pilon, 2014). Heterocysts are the site for nitrogen fixation and they are formed when the nitrogen sources are depleted. Akinetes are more resistant to