The Effects of pH and Cell Density to the Responses of Immobilized Cyanobacteria for Copper Detection

Rubban Kunasakaran, Sughan Vijayan, and Ling Shing Wong
Faculty of Science, Technology, Engineering and Mathematics/INTI International University, Persiaran Perdana BBN, 71800 Nilai, Negeri Sembilan, Malaysia
Email: {rubbhan, sughanv} @gmail.com; lingshing79@yahoo.com.sg

Mee Kin Chai
College of Engineering/Universiti Tenaga National, Jalan IKRAM-UNITEN, 43000 Kajang, Selangor, Malaysia
Email: mkchai@uniten.edu.my

***Abstract*—Rapid urbanization and industrialization lead to heavy metal pollution which poses a serious threat to human's health. Thus, there is a continuing need to develop a device that can be easily used to monitor and detect the presence of heavy metal pollution. Whole cells e.g. cyanobacteria have been reported as potential bio-indicators for environmental heavy metals detection. However, the intensity of the biological responses of these cells is affected by pH and the cell density. In this paper, the effect of pH and cell density to the response of cyanobacteria *Anabaena cylindrica* to copper (Cu) is reported. The cyanobacteria were cultured and immobilized with agarose in cuvettes before the exposure to Cu solution (1.0 mg/L). The measurement was carried out with OD= 680 nm using a spectrophotometer, which indicated the changes of chlorophyll. The most significant response of the cells was measured at pH 8 and the optimal density of the cell for the detection was 500,000 cell/mL. This study was an important stage to determine the potential of *A. cylindrica* to be used in heavy metal detection.**

***Index Terms*—bio-indicator, heavy metal toxicity, cyanobacteria, pH, cell density**

I. Introduction

Heavy metals are naturally occurring elements which can be found in the environment [1]. The high concentration of these elements will lead to toxicity effect to the living organisms [2], [3]. The heavy metals have to be constantly monitored in order to improve the quality of environment and the safety of human beings.

Whole cells can be used as bio-indicators to detect the presence of heavy metals [4]-[7]. Some of the cells are highly sensitive, relatively inexpensive, response rapidly, and can be used for continuous monitoring of heavy metals in the environment [8], [9]. One of the main components of a bio-indicator is the bio-recognition element. In this work, cyanobacteria *A. cylindrica* had been used as the bio-recognition element. Cyanobacteria *Anabaena* strains contain photosynthetic pigments such as chlorophyll a and carotenoids [10]-[12]. These pigments can be used as bio-recognition elements as they produce biological signals which can be captured using electronic transducer after being exposed to various heavy metals.

The pH value is known as the potential of hydrogen. It is a measurement of the concentration of hydrogen ions in the solution [13]. The pH of pure water is 7 which is the neutral condition and a value lower than 7 indicates acidic; whereas, higher than 7 indicates alkaline. The pH influences the solubility and availability of certain solutes such as nutrients [14], [15]. Difference in pH value can give a detrimental effect on the growth of marine organism including algae. There are several ways pH can affect the algae such as changing the distribution of carbon dioxide in the water, and the availability of carbon as an energy source for the cells. Furthermore, the effect of pH level also has been studied on its direct influence on physiological characters of algae in changing the trace metals availability. On the other hand, different density of cells might affect the intensity of cells' response to the heavy metals [16]. Therefore, it is important to determine the optimum cell density in order to yield the best signal output for that particular biosensor.

In this paper, the usage of immobilized cyanobacteria *A. cylindrica* for heavy metal detection is described. The immobilization enabled the exposure of the cells to the heavy metal solution with better stability and increased the performance of the bio-indicators [16], [17]. Although chlorophyll in cyanobacteria has been widely reported as a potential reporter in biosensor applications, most of the researches were focused on the fluorescence response of chlorophyll and the changes of oxygen produced as measuring parameters [10], [18]-[21]. The spectrophotometry design by measuring optical density (OD) at λ = 680 nm has not been reported before. The objectives of this research were to determine the effect of pH and cell density towards the responses of *A. cylindrica* to Cu, with the changes in OD selected as measuring parameter.

Manuscript received September 23, 2014; revised December 2, 2014.

doi: 10.12720/jolst.2.2.78-81

II. Methodology

A. The Design of the Test

The design of *A. cylindrica* as bio-indicator is shown in Fig. 1. The immobilization using agarose was carried out by mixing 0.5 mL of agarose solution (1 %) with 0.5 mL of *A. cylindrica* cells in a cuvette. The mixture was left to solidify for few minutes on the bench on the clear side of the cuvette.

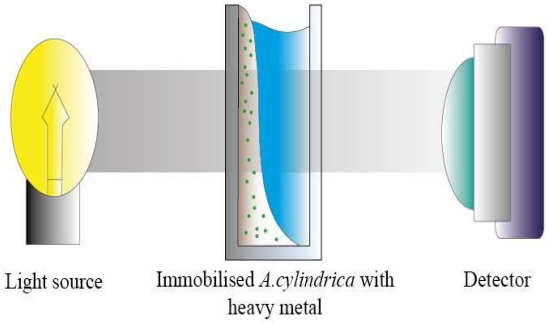


Figure 1. Overview mechanism of *A. cylindrica* bio-indicator

B. The Effect of pH to the Cells

1 mg/L of Cu solution prepared. The pH of the solutions was adjusted with NaOH and HCl to the pH values of 6, 7, and 8 respectively. The measurement of pH value was carried out with pH meter (Starter 3000, Ohaus). 2 mL of Cu solution with the pH 6 was added to the immobilized cells, and the OD was measured at 0 hour, 1 hour, 6 hours, and 24 hours after the exposure. These steps were repeated for the pH 7 and pH 8 of Cu respectively. The reading produced by the immobilized cyanobacteria exposed to 2 mL of distilled water (pH 7) without Cu was served as negative control, while the cuvette with distilled water was served as blank. The absorbance readings were measured at wavelength 680 nm using UV-vis spectrometer (GeneQuant 1300, GE). All the tests were conducted with n = 6 unless stated otherwise.

C. Preparation of Solution with Different Cell Concentration

Cultures with cell densities of 1.0 x 10^6 cell/mL, 7.5 x 10^5 cell/mL, 5.0 x 10^5 cell/mL, 2.5 x 10^5 cell/mL and 1.0 x 10^5 cell/mL were prepared and immobilized with agarose. The immobilized cells were exposed to the 1 mg/mL solution respectively.

D. Analysis of Data

The average values of the OD and its standard deviations were calculated using Microsoft Excel 2010. The percentage of OD change was calculated using the following equation:

$$\% I = (Io-I)/Io \times 100\%$$

where,

Io = OD change before Cu

I = OD change after Cu

III. Results and Discussion

A. The Effect of pH to the Response of A. cylindrica

This experiment explored the effect of different pH of Cu solution on the response of *A. cylindrica*. The pH used was pH 6, pH 7, and pH 8 which represented acidic, neutral and alkaline condition correspondingly. Fig. 2 shows the effect of Cu (1 mg/L) in three different pH values to the cells. The percentage of OD change has been recorded at four different time of exposure. The overall results show the OD decreases over a period of 24 hours and pH 8 gave the best response, by reaching a stable state around 6 hours of exposure. The exposure time of 24 hours showed best stability with pH variations gave lesser changes to the OD.

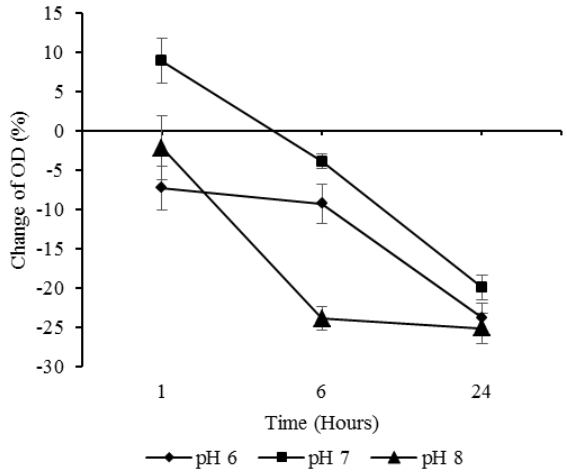


Figure 2. The change of OD (λ = 680 nm) with immobilized *A. cylindrica* exposed to different pH of Cu for 24 hours. The graph was plotted with the negative control served as base-line

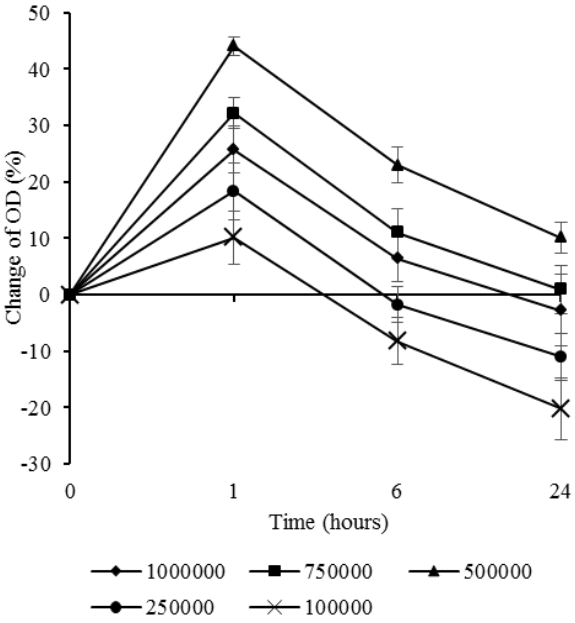


Figure 3. The OD changes with different cell concentrations for 24 hours of exposure to Cu

This study showed that the cells gave better responses at pH 8 over 24 hours' time. The results were in a good agreement with the previously done studies on effect of

pH for cyanobacteria growth which also gave a good response to pH 8 [3]. However, it is important to note that the effect of pH is also species dependent, especially for cyanobacteria. As an example, cyanobacteria *Spirulina major* gave good response in pH 6.5. Another experiment carried out by Giraldez-Ruiz and Mateo [15] confirmed that *Anabaena* was not suitable to be cultured with pH below 6. The same study suggested that acidic condition was not suitable for *Anabaena* as the internal pH control would be lost due to the low pH environment.

B. The Effect of Cell Densities to the Response of A. cylindrica

A. cylindrica was found giving response with cell density ranging from 1 x 10^7 cell/mL to 1 x 10^5 cell/mL. The cell-density dependant responses were well described by Wong *et al.* [16] and Kumar *et al.* [22], which the cell densities exceeding or below the optimum number decrease the response produced. Fig. 3 shows the effect of cell density of *A. cylindrica* to the response of the cells, tested with 1 mg/L of Cu concentration at pH 7. The OD measured for 24 hours indicated the responses of *A. cylindrica* to Cu were related to the density of the cell used.

The increase in OD for all the cells' density in the first hour of exposure was mainly due to a small amount of Cu uptake by the cells in a short period of time. In addition to that, copper acts as a trace element which involves in removal of superoxide radicals and electron transfer during photosynthesis [2]. On the other hand, biochemical mechanism of the cyanobacteria to mediate the effect of Cu might be another cause to it. Most cyanobacteria cells produce metallothionein which will bind to the heavy metal to detoxify them, homeostasis and defence against oxidative stress [23]. The OD readings started to drop after 1 hour of exposure to Cu, as the excessive uptake of Cu exhibited toxicity effect to the cells.

IV. Conclusions

In this research, the effect of pH and the effect of cell density to the response of *A. cylindrica* to Cu have been studied. Although further research is required, a clear trend of variation in OD caused by the changes of pH and cell density was the evidence. The results from this research are important references in bio-indicator development using the cyanobacteria as the biological component.

Acknowledgment

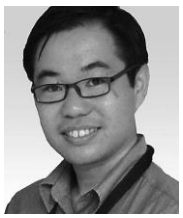
This project was funded by INTI International University, Malaysia, with grant no. INT-FOSTEM-03-01-2014.

References

[1] E. Callender, "Heavy metals in the environment-historical trends," *Treatise on Geochemistry*, vol. 9, pp. 67-105, 2003.
[2] A. Badarau and C. Dennison, "Thermodynamics of copper and zinc distribution in the cyanobacterium synechocystis PCC 6803," *Proceedings of the National Academy of Sciences*, vol. 108, pp. 13007-13012, 2011.
[3] A. Bano and P. J. Siddiqui, "Characterization of five marine cyanobacterial species with respect to their pH and salinity requirements," *Pakistan Journal of Botany*, vol. 36, pp. 133-144, 2004.
[4] A. Berezhetskyy, *et al.*, "Conductometric biosensor based on whole-cell microalgae for assessment of heavy metals in wastewater," *Biopolimery I Kletka*, vol. 23, p. 511, 2007.
[5] S. I. Choe, *et al.*, "Role of aspergillus niger acra in arsenic resistance and its use as the basis for an arsenic biosensor," *Applied and Environmental Microbiology*, vol. 78, pp. 3855-3863, 2012.
[6] C. M. Zammit, *et al.*, "A whole-cell biosensor for the detection of gold," *PloS One*, vol. 8, pp. e69292, 2013.
[7] K. Yoshida, *et al.*, "Novel carotenoid-based biosensor for simple visual detection of arsenite: characterization and preliminary evaluation for environmental application," *Applied and Environmental Microbiology*, vol. 74, pp. 6730-6738, 2008.
[8] J. Castillo, *et al.*, "Biosensors for life quality: Design, development and applications," *Sensors and Actuators B: Chemical*, vol. 102, pp. 179-194, 2004.
[9] W. N. A. Wan Jusoh and L. S. Wong, "Exploring the potential of whole cell biosensor: A review in environmental applications," *International Journal of Chemical, Environmental & Biological Sciences*, vol. 2, pp. 52-56, 2014.
[10] W. L. Shing, *et al.*, "The fluorometric response of cyanobateria to short exposure of heavy metal," *Advances in Environmental Biology*, vol. 6, pp. 103-108, 2012.
[11] P. Fay, "Cell differentiation and pigment composition in anabaena cylindrica," *Archiv für Mikrobiologie*, vol. 67, pp. 62-70, 1969.
[12] S. Hertzberg, *et al.*, "The carotenoids of blue-green algae," *Phytochemistry*, vol. 10, pp. 3121-3127, 1971.
[13] A. K. Covington, *et al.*, "Definition of pH scales, standard reference values, measurement of pH and related terminology (Recommendations 1984)," *Pure and Applied Chemistry*, vol. 57, pp. 531-542, 1985.
[14] C. Y. Chen and E. G. Durbin, "Effects of pH on the growth and carbon uptake of marine-phytoplankton," *Marine Ecology Progress Series*, vol. 109, pp. 83-94, 1994.
[15] N. Giraldez-Ruiz, *et al.*, "The relationship between intracellular pH, growth characteristics and calcium in the cyanobacterium anabaena sp. strain PCC7120 exposed to low pH," *New Phytologist*, vol. 137, pp. 599-605, 1997.
[16] L. S. Wong, *et al.*, "Performance of a cyanobacteria whole cell-based fluorescence biosensor for heavy metal and pesticide detection," *Sensors*, vol. 13, pp. 6394-6404, 2013.
[17] S. D'souza, "Microbial biosensors," *Biosensors and Bioelectronics*, vol. 16, pp. 337-353, 2001.
[18] T. C. Chay, *et al.*, "A copper toxicity biosensor using immobilized cyanobacteria, *Anabaena torulosa*," *Sensor Letters*, *3*, vol. 1, pp. 49-54, 2005.
[19] L. S. Wong, *et al.*, "Whole cell biosensor using anabaena torulosa with optical transduction for environmental toxicity evaluation," *Journal of Sensors*, vol. 2013, 2013.
[20] D. Merz, *et al.*, "Chlorophyll fluorescence biosensor for the detection of herbicides," *Fresenius' Journal of Analytical Chemistry*, vol. 354, pp. 299-305, 1996.
[21] H. Nguyen-Ngoc and C. Tran-Minh, "Fluorescent biosensor using whole cells in an inorganic translucent matrix," *Analytica Chimica Acta*, vol. 583, pp. 161-165, 2007.
[22] J. Kumar, *et al.*, "Optical microbial biosensor for detection of methyl parathion pesticide using flavobacterium sp. whole cells adsorbed on glass fiber filters as disposable biocomponent," *Biosensors and Bioelectronics*, vol. 21, pp. 2100-2105, 2006.
[23] V. Hassinen, *et al.*, "Plant metallothioneins–metal chelators with ROS scavenging activity?" *Plant Biology*, vol. 13, pp. 225-232, 2011.

Rubban Kunasakaran was born on 8th May 1990 at Selangor, Malaysia. He is qualified with Bachelor of Science in Biotechnology at Inti International University. His passion towards science has encouraged him to create a biosensor device incorporate with smartphone app and for that he won a silver medal at International Innovation Festival 2014. Also due to his enthusiasm towards academic, Rubban has obtained four certificates as well as three diplomas in various field of expert through online studies. Lastly, Rubban has published his self-renowned smartphone game at Google Play.

Sughan Vijayan was born on 20th May 1987 in Selangor, Malaysia and qualified with his degree in Bachelor of Science of Biotechnology at Inti International University Nilai, Malaysia. He has worked as an intern at Geneflux Biosciences Sdn Bhd in 2010 and he also has obtained a certificate as a Micro Technician assembly from Cosmopoint Institute of Information Technology in 2005.



Wong Ling Shing graduated from The National University of Malaysia (UKM), Bangi, Selangor, Malaysia in 2010 with Ph.D. in Environmental Science, specialized in the development of biosensor for environmental toxicity assessment, and bio-analytical chemistry. He obtained his M.Sc. with the major in Conservation Biology and B.Sc. (Hons) in Biochemistry from the same university.He is currently a senior lecturer at Inti International University, Nilai, Negeri Sembilan, Malaysia. His research interests include development of biosensor for environmental application, discovery of new reporter groups for biosensor applications, cell culture, and polymer. He has authored and co-authered more than 35 scientific articles. He extended his service as editorial member in Advanced Biological Chemistry, Asian Journal of Natural and Applied Sciences, and European Journal of Natural and Applied Sciences, and serves as active reviewer for several other journals. Dr. Wong is a senior member of Asia-Pacific Chemical, Biological & Environmental Engineering Society (APCBEES) and the Institute of Research Engineers & Doctors (theIRED).

Mee Kin Chai was born in Perak, Malaysia, on May 1974. She received the BSc. and MSc. degrees in chemistry from the Universiti Teknologi Malaysia, Skudai, Johor, Malaysia in 1998 and 2000, respectively and the Ph.D. degree in analytical chemistry the Universiti Malaya, Kuala Lumpur, Malaysia in 2009. In 2000, she joined the Department of Engineering Science and Mathematics, College of Engineering, Universiti Tenaga Nasional, as a Lecturer, and in 2009 became a Senior Lecturer. She is currently a Senior Lecturer at Department of Civil Engineering, College of Engineering, Universiti Tenaga Nasional, Malaysia. She has published more than 40 scientific papers and books in the field of green analytical chemistry. Her current research interests include applying the green chemistry in the determination of trace organic in the environmental samples, method development for analyzing and characteristics of chemical compounds, composition, structure, relationships and reactions utilizing modern green analytical techniques.Dr. Chai is a member of the Malaysian Institute of Chemistry and Malaysian Analysis Chemistry Society. She is an active external reviewer for international Journal of Food and Chemical Toxicology (Elsevier), Food Chemistry (Elsevier) and international Journal of Environmental analytical chemistry (Taylor & Francis Group).