CAROTENOIDS AS BIOREPORTER FOR ENVIRONMENTAL TOXICANTS ASSESSMENT: A SPECTROMETRY APPROACH

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Abstract- Environmental pollution is a global crisis due to the anthropogenic activities. Conventional chemical analytical tools and biosensors are effective tools in environmental toxicant assessment. For whole cell biosensors, chlorophyll was a well-studied pigment as bioreporter for the presence of environmental toxicants, but other pigments were not widely reported. In this paper, the usage of another widespread photosynthetic pigments- carotenoids as bioreporter of environmental toxicant is discussed through a few possible biosensor designs, with the sensitivity, reproducibility and storability evaluated. The carotenoids are found to be good candidate as bioreporters as the pigments response was acceptable with dosage dependency. However, the storability was still an issue which a better immobilization or storage condition was the key to a better results.

Keywords- Carotenoids, environmental pollutants, bioindicator, photosynthetic organisms.

I. INTRODUCTION

Environmental pollution is a global crisis. Anthropogenic activities, such as the development in agriculture and industry, as well as the dumping of domestic wastes have released hazardous compounds into environment.

Two types of most commonly knew pollutants are the metals and pesticides. Metals e.g. lead (Pb), cadmium (Cd), nickel (Ni), aluminium (Al), are poisonous to the floras and faunas, and will devastate humans' health through direct contact or the food chain [1-4]. Pesticides e.g. 2,4-D and atrazine are useful in agriculture but at the same time, bring might bring negative impact to the environment and human in case of misuse [5, 6].

To date, many methods have been established to indicate the presence of these pollutants in environment, e.g. AAS and HPLS [7, 8]. However, the widespread of these pollutants makes the detection and monitoring work overwhelming. Many bio-sensing devises are developed and proved to be effective in the assessment effort [9-12]. These screening-effective devises might be useful with the combination of conventional chemical analytical tools. In whole cell biosensors, chlorophyll has been identified as one of the effective reporter for heavy metals and pesticides detection [13]. As a key component in photosynthesis, chlorophyll-related responses, such as the change in the level of oxygen, fluorescence, and the content of the pigment itself have been fully utilized in the screening of environmental pollutants [14-16].

However, the potential of other photosynthetic pigments e.g. carotenoids and phycocyanin attracted less attention. In this article, the potential of one of the photosynthetic pigments- carotenoids is discussed along with the results obtained through a series of experiments conducted on different sources of carotenoids.

II. CAROTENOIDS AS BIOREPORTER

Carotenoids produce yellowish to red colours in photosynthetic organisms. These pigments are important as light harvester while protecting these organisms from reactive oxygen species [17, 18]. As light harvesting pigments, carotenoids can be detected with spectrophotometer at absorption wavelength between 400 nm to 480 nm. The recent research showed the potential of carotenoids contained in Daucus carota cells coupled with spectrometry detection in heavy metals assessment [19]. The results then was consolidated by the follow up experiments, which confirmed the change in carotenoids content in the cells after the exposure to copper (Cu), Pb, and zinc (Zn), and effects of several factors e.g. pH, cell density, and the immobilization condition to the response were determined [20, 21]. The exposure of cyanobacteria to heavy metals recorded the change in carotenoids as well [22]. The immobilized Anabaena cylindrica showed sensitivity to Cu and Pb. The effect of cell age and density were found affecting the responses of the cyanobacteria.The design of a biopolymer entrapped carotenoids for the detection of heavy metals used the formation of biopolymer matrix to entrap the pigments, which the pigments reacted with the analyte solution to form emulsion at different rate and intensity, and brought changes of OD at the wavelength 450 nm [10].

III. SENSITIVITY

A current research conducted confirmed good sensitivity of carotenoids towards metal ions. The D. carota cells used were immobilized by agarose and the change in carotenoids were indicated by spectrometry approach [20]. Linear detection ranges for both Pb and Cu tests were within 0.01 – 10.00 mg/L, with the lowest detection limit of 0.01 mg/L

for both metals. The experiment showed the responses of cells were affected by the density of the cells, as well as the pH of the sample and the cell age. The plant cell showed higher sensitivity towards Cu exposure. Similar research was conducted with D. carota free cells yielded the identical trend of responses from carotenoids after the exposure to Cu, Pb, and Zn [21]. Another research done using naturally occurring carotenoids in cyanobacteria Anabaena cylindrica produced considerably good results [23], where the lab cultured cyanobacteria was immobilized and exposed to Cu and Pb, with the linear detection ranges of 0.01 – 10.00 mg/L for both metals. The experiment showed the cells from different growth phases could be potentially used for the metals detection as well. Similar to the plants cells, the cyanobacteria was more sensitive to Cu compared to Pb. When the research extended to the utilization of carotenoids in biopolymer biosensor, a promising results in the detection of Cu, Pb, Zn, and Al are produced, with majority of the detection ranges fall within 0.01 – 10.00 mg/L [10]. The sensitivity of a bioindicator can be defined from the slope of the linear detection range. As described by Wong and Wong [10], the carotenoids entrapped in biopolymer were most sensitive to Pb, followed by Zn, Al, and Cu. The carotenoids in free D. carota cells were found most sensitive to Pb, followed by Zn and Cu [21]. Immobilized D. carota however showed no significant difference in sensitivity between Cu and Pb [20]. Among different design and immobilization of bioindicator, the exposure time for the carotenoids in biopolymer was the shortest at 15 minutes, while the exposure time of immobilized D. carota was found optimized at 75 minutes. The exposure time for the immobilized cyanobacteria A. cylindrica was however, was 24 hours, which might not be effective for rapid detection. Although tests conducted showed D. carota free cells responded to metals within 20 minutes of exposure, the free cells might decrease the portability and storability, thus reduce the practicality as biosensor.

Table 1. Bioindicators using carotenoids for environmental applications

Source of carotenoids	Pollutants	Lower limit of detection mg/L	Linear detection range (approx.) mg/L	Ref.
D. carota free cells	Cu	0.01	N.A.	[21]
	Pb	0.01	N.A.	
	Zn	0.01	N.A.	
Immobilized *D. carota*	Pb	0.01	0.01 – 10.00	[20]
	Cu	0.01	0.01 – 10.00	
Immobilized *A.cylindrica*	Cu	0.01	0.01 – 10.00	[23]
	Pb	0.01	0.01 – 10.00	
Palm oil entrapped by biopolymer	Cu	0.01	0.01 – 10.00	[10]
	Pb	0.01	0.01 – 10.00	
	Zn	0.01	0.01 – 10.00	
	Al	0.10	0.10 – 10.00	

III. REPRODUCIBILITY AND STORABILITY

Reproducibility and storability are important in defining the practicality of a bioindicator, especially for the biosensing applications. Immobilized D. carota had good reproducibility with a standard deviation less than one percent (< ± 1%) for all the exposure tests, with n = 3 [20]. However, the storability test for 40 days exhibited significant reduce in response, especially in the first 10 days of storage. The test with D. carota free cells showed lower reproducibility of approximately ± 2 – 5 % of standard deviation [21]. The results suggested that the immobilization brought the biological component closer to the transducer and stabilized the transmission of biological signals to the transducer. Due to the nature of the culture, the storability of the D. carota free cells couldn't be conducted.

For carotenoids in biopolymer, the responses to all metals tested showed considerably good reproducibility of ± 0.2 – 2.8 %. The good reproducibility showed immobilization was a good practice to yield better performance, which was agreeable by several previous researches on biosensors development [9, 20, 24].

IV. FUTURE DEVELOPMENT

The research on the change of carotenoids to the presence of environmental pollutants will be extended to the photosynthetic organisms collected from the natural environment. Currently, experiment on Spirogyra collected showed promising carotenoids responses to two types of pesticides. This research opened the door of utilizing native photosynthetic organisms for in situ detection of the pollutants. The responses of carotenoids to one of the pesticides, atrazine is depicted in Figure 1. The results are complemented by another research conducted using consortium of algae and cyanobacteria collected from fish culturing pond showed similar results with carotenoids in these consortium changed after being exposed to environmental pollutants (Figure 2).

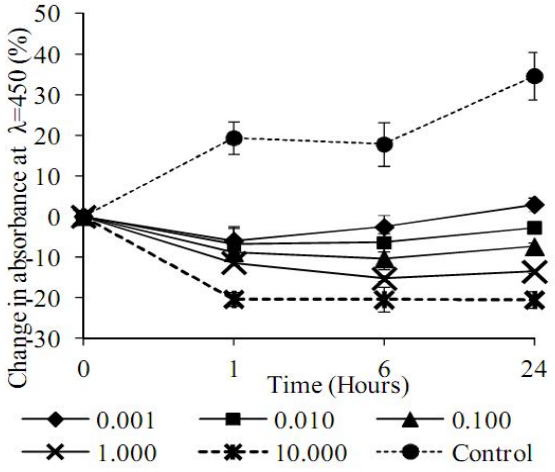

Figure 1. The responses of carotenoids to different concentrations of atrazine at different exposure time.

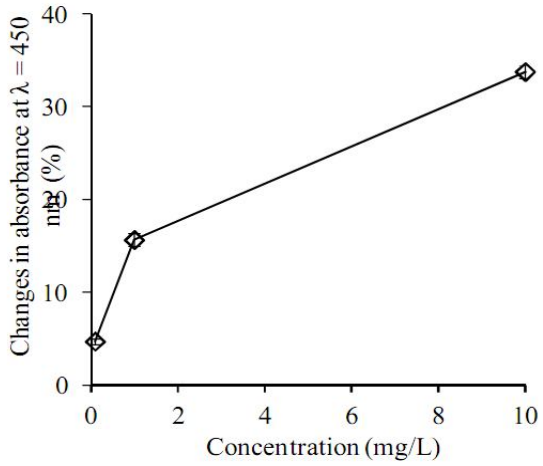

Figure 2. The responses of carotenoids in consortium to different concentration of Pb.

Storability is always an issue for cell biosensors, as the performance of the biosensors will decrease together with the degradation of the cells. Cell biosensors could useful for screening all types of pollutants, without elucidating the type of the pollutants. This can be an advantage for the cells to act as first level of indicator before the suspected sample being sent to the lab for further analysis.

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