Rapid Detection of Heavy Metals with the Response of Carotenoids in *Daucus Carota*

Ling Shing Wong and Chieh Wean Choong

***Abstract*—In this paper, a rapid detection method for heavy metals (Cu, Pb and Zn) is reported. The method was based on the response of natural cell-bounded carotenoids in *Daucus carota* to short term exposure of heavy metals. The cells were cultured for 90 days in dark. The presence of carotenoids in cells was confirmed spectrometrically with optical density (OD) measured at λ= 450 nm. The responses of the cells to 0.01, 0.10, and 1.00 ppm of heavy metals were confirmed by the increase of OD after the short term exposure, with the lowest detection were recorded at 0.01, 0.10, and 0.01 ppm respectively. The OD increase might be a result of the synthesis of carotenoids, which was probably triggered by the increase of oxidative stress. With an average optimum response time of 40 minutes, the cell could be potentially utilized in biosensor.**

***Index Terms*—Biosensor, *Daucus carota*, natural cell-bounded carotenoids, heavy metal.**

I. Introduction

Pollution is a worldwide crisis. Due to the overwhelming number of sample analysis and practical consideration, many scientific analysis tools have been developed for rapid detection of pollutants.

Whole cell biosensors offer an alternative way of detection by utilizing biochemical responses of cell towards pollutants such as heavy metals. The biochemical responses can be detected by transducers and then transformed into digital outputs. The correlation between the biochemical responses and digital outputs can be used to detect the heavy metals, either quantitatively, or qualitatively.

So far, detection of biochemical responses such as the chlorophyll fluorescence [1]-[3], oxygen production, enzyme activities [4], and bioluminescence have been reported. The colour pigment which has been extensively studied is the chlorophyll. As a light harvesting pigment, the inhibition of photo-phosphorylation by heavy metals will result in the increase of fluorescence [5], [6]. However, utilization of other types of widely available natural cell-bounded pigments such as carotenoids in biosensor application is lacking.

Carotenoids are linear polyenes which can be synthesized by many photosynthetic organisms and therefore the most widely distributed accessory pigments [7]. In deciduous trees, the pigments are responsible for the yellowish to red fall colours, as well as for the colour of carrots. In plants, carotenoids serves as light harvesting pigments, which have excellent absorption of the wavelength between 400 nm to 480 nm. Besides, carotenoids help to reduce the production of reactive oxygen species by absorbing the energy from chlorophyll and dissipate the energy through internal conversion as heat [8]. In the context of reducing the reactive oxygen species, carotenoids serves as non-enzymatic antioxidants which protect the plants from the destruction of the oxidative stress [9].

Heavy metals have been reported as photosynthesis inhibitors [10]-[14] by uncoupling the oxidation-reduction sites in photosystems. The presence of heavy metals increases the amount of reactive oxygen species and at the same time increase oxidative stress in plants [15]. Thus, the reaction of natural cell-bounded carotenoids to the exposure of heavy metals is well anticipated.

Dar *et al.* [16] reported that carotenoids content of *Triticum aestivum* L. reduced after being exposed to heavy metals for three. On the other hand, Pinto *et al.* [17] reported that the exposure of heavy metals to *Gracilaria tenuistipitata* for 6 induced the synthesis of carotenoids. The response has been utilized in environmental pollutants detection. Rahman *et al.* [18] depicted a possible way using cyanobacteria contained carotenoids in biosensor application, with the incubation time of 10 days. Yoshida *et al.* [19], [20] utilized the production of carotenoids in mutated purple bacteria *Rhodopseudomonas palustris* no. 7 in heavy metal detection, with the increase of carotenoids detected in 6 hours of inhibition. However, the practicality of these applications has been limited either by the usage of transgenic organisms or the long exposure time required to enable the detection.

In this paper, the responses of carotenoids contained in *D. carota* cells in suspension to Cu, Pb and Zn are reported. The responses could be detected within 60 minutes of exposure to heavy metals, which was faster than previously reported.

II. Methodology

A. Chemicals and Glassware

The chemicals used for the preparation of MS medium were purchased from R & M Marketing, Sigma-Aldrich, Systerm, and Phyto Technology Laboratories. All the glassware used in this experiment was autoclaved with 121 ℃ and 15 psi for 15 minutes. All the equipment used in cell cultured was sterilized with 70% ethanol before use.

B. Cell Preparation

D. carota taproot was sliced into discs with thickness of approximately 1 cm, immersed in 70% ethanol for 30 s and agitation in sterilant (0.525% NaOCl, 0.05% Triton X-100) for 25 minutes. It was rinsed thrice with distilled water

Manuscript received July 1, 2013; revised November 3, 2013.

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afterward. The discs were excised with cambium layer in the middle. These processes were conducted in sterile Laminar Flow Cabinet (ESCO).

These explants were cultured on MS medium [21] with 25 g/L sucrose, 2.2 g/L gelrite and 1 mg/ml 2, 4-dichlorophenoxyacetic acid. The culture was maintained in dark condition for 60 days in the culture room.

The explants were sub-cultured after 60 days and incubation at 25℃ for in dark for 30 days. The resulting liquid with cell suspension was filtered to minimize the number of clumps. Centrifugation was conducted using Heraeus Megafuge 10R (Thermo Electron Corporation) to collect the cells. The cell pellet was resuspend in MS broth medium and ready to be used.

C. The Absorbance of Carotenoids

A volume of 2 mL of *D. carota* cell suspension was transferred into a cuvette, followed by spectrophotometry determination of optimized wavelength, as described by Wong and Choong [22]. The wavelength with the highest OD was identified at 450 nm. The wavelength was used to determine the change in carotenoids content in the experiment.

D. Heavy Metals Detection

The stock solutions of Cu with concentrations of 10.0 ppm and 0.1 ppm were prepared. Other solutions concentrations (0.01 and 1.00 ppm) of Cu solution used in the experiment were prepared by the dilution of the stock solutions.

To study the responses of the cells to 1 ppm of Cu, 0.2 mL of 10 ppm Cu stock solution was transferred into 1.8 mL medium containing *D. carota* cell suspension, to make a final concentration of 1 ppm of Cu. OD readings were taken before the exposure. OD readings were taken again after 20, 40 and 60 minutes of exposure to Cu. The experiment was repeated using Cu with concentration of 0.10 ppm and 0.01 ppm respectively in triplicate.

The same procedure was then applied to the tests on Pb and Zn. All the experiments on heavy metals detection were conducted in triplicates.

E. Data Analysis

The percentage of OD increment was calculated as follows:

Percentage (%) of OD increment =
$[(OD_1 – OD_0) / OD_0] \times 100\%$

where,

OD_0= OD before the exposure to heavy metals

OD_1= OD after the exposure to heavy metals

The data obtained from responses of *D. carota* to heavy metals over 60 minutes of exposure were compared to the responses of cells without heavy metals (blank, with 1.8 mL of cell culture and 0.2 mL of distilled water). All the analysis in this experiment was conducted using Microsoft Excel.

III. Results and Discussion

Spectrometry analysis on *D. carota* cells showed highest absorbance at 450 nm, which was in agreement with the absorbance of carotenoids [23], [24], thus the presence of carotenoids was evidence. The culture of taproot cell was conducted in dark condition, in order to promote the synthesis of carotenoids, on the other hand inhibiting the synthesis of chlorophylls [25], [26].

The response of carotenoids to Cu is illustrated in Fig. 1. The exposure raised the absorbance for all concentration of Cu tested. The maximum OD was yielded at 40 minutes of exposures.

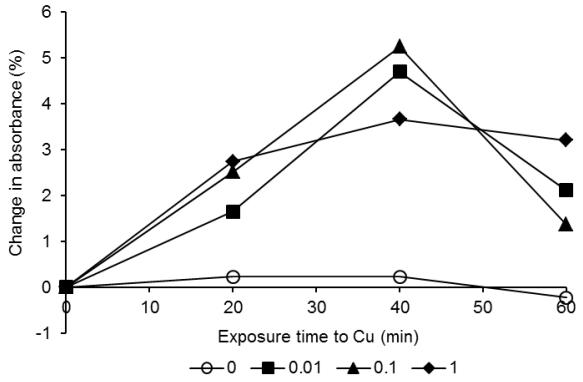

Fig. 1. Response of carotenoids as indicated by the percentage of change in absorbance over time at various concentration of Cu.

D. carota bounded carotenoids showed similar trend to the exposure tests on Pb and Zn, as depicted in Fig. 2 and Fig. 3. Maximum absorbance yielded predominately at 40 minutes. The cell showed observable response to Cu and Zn tested at 0.01 ppm. However, the response of carotenoids to Pb 0.01 ppm has no significant difference from blank. This indicated that the sensitivity of the cell to Pb was lower than Cu and Zn. The result was different from the research done on cyanobacteria by Wong *et al.* [2] showing the photosynthetic organism was more sensitivity to Pb than Cu.

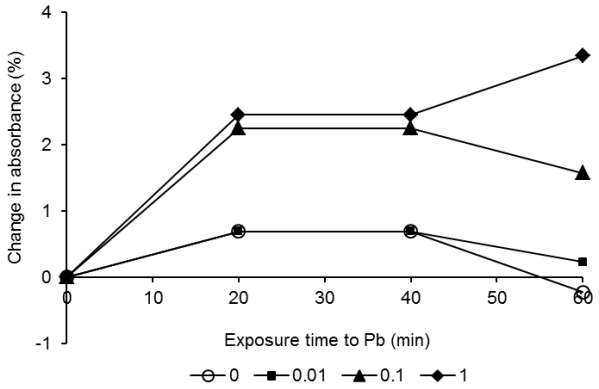

Fig. 2. Response of carotenoids as indicated by the percentage of change in absorbance over time at various concentration of Pb.

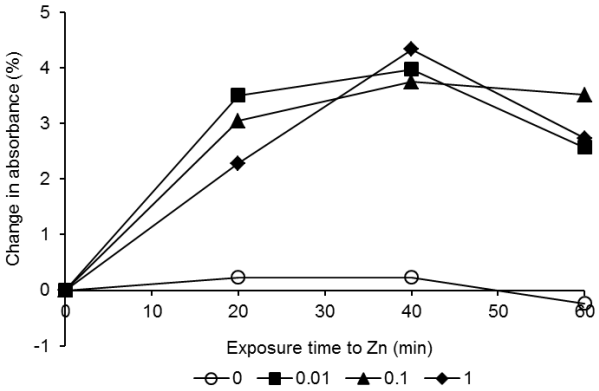

Fig. 3. Response of carotenoids as indicated by the percentage of change in absorbance over time at various concentration of Zn.

Cu is a micronutrient which is essential to photosynthetic organisms in small amount in ionic form. In plants, Cu is required in metalloprotein synthesis. Metalloprotein is an important element in electron transfer of photosynthetic redox [27]. However, Cu will affect cell's metabolism in high concentration [28]. Pb is non-beneficial and non-functional element for photosynthetic organisms. Low concentration of Pb will trigger the toxicity effect where inhibition of photosynthesis is evidence [29], [30]. Thus, the toxicity effect of Cu is always expected to be lower than Pb. However, *D. carota* cell used in this experiment responded otherwise.

The responses of the cell on 40 minutes of exposure to various concentrations of heavy metals after noise filtration are shown in Fig. 4. Although Cu induced the highest response with a glance, statistical test showed no significant difference ($p < 0.05$) between Cu and Pb, as well as Zn and Pb.

The biosensors constructed for environmental application needs to be sensitive and able to detect analytes in short period of time [31]. In this experiment, the cell showed good response towards heavy metals. The cell was able to detect 0.01 – 1.00 ppm of Cu and Zn, as well as 0.10 – 1.00 ppm of Pb. The short response time of 40 minutes was a good indication that the cell could be used as biological component in a biosensor. Average standard deviations for the tests on Cu, Pb, and Zn were ±4.90%, ±4.26%, and ±1.95% respectively (n= 3).

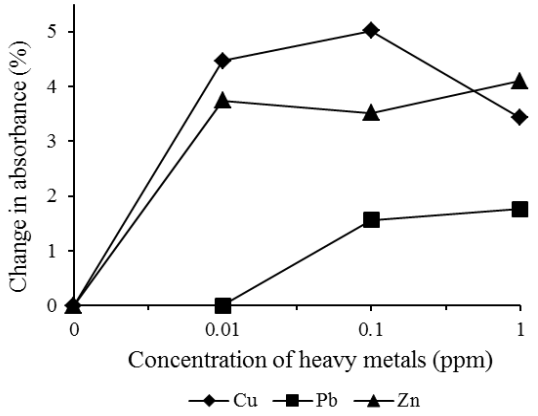

Fig. 4. Response of carotenoids as indicated by absorbance at 450 nm against various concentrations of heavy metals (Cu, Pb and Zn) at 40 minutes exposure time.

Although the cell showed good sensitivity and reproducibility in the tests with a few types of heavy metals, further work is required to incorporate the cell into a biosensor. The factors which influence the response of cell, such as the condition of the culture, the number of cell to be used in detection, the detection condition such as pH are yet to be studied [2], [3], [32]. The cells cultured respond differently in different growth stage, while different number of cells used affects the signal produced as well. According Wong *et al.* [5], the increase of the signal produced was positively proportionate to the number of cells used within a limit, whereas the increase of cells used after the limit diminished the signal produced.

The immobilization of cells will be tested as it is an important process in biosensor construction, which helps to increase the performance of biosensor by bringing the biocomponent closer to the transducer [3], [5]. The reports show that chitosan and acrylamide [33], poly-2-hidroxy-ethyl-methacrylate [5], alginate and silica [34], and agarose [35] have been utilized in biosensor construction as immobilizing agents. This work will be continued by immobilization of *D. carota* cells on a suitable support medium by a selected immobilizing agent. Agarose might be a good candidate for its natural derived composition and the ability to polymerize under low temperature.

The operation environment of the biosensors affects the respond of the cells, which has to be studied as well. pH is most widely studied factor, as bioconponents are affected by the pH of the environment [32], [36].

Besides, the stability of immobilized cells will have to be tested. The stability of biosensors is important feature to determine the storability of the biocomponent over a period of time. It is a normal trend where biocomponent deteriorates over a period of storage time [5], [36]. The higher the stability, the better the biosensor retains the signal produced over a period of time of storage. The storability reflects the practicality of the biosensor as well.

IV. Conclusion

The spectrometric response of *D. carota* to heavy metals at the absorbance of 450 nm showed that carotenoids can be used as biological element in biosensor, with the detection limits at 0.01 – 0.1 ppm and the response time of 40 minutes. However, more work has to be done in order to fully understand the mechanism behind the increment of absorbance at 450 nm.

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