

Naturally Occurring Carotenoids in Cyanobacteria as Bioindicator for Heavy Metals Detection

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Abstract—Heavy metals pollution is a worldwide issue which requires close monitoring. The presence of naturally occurring cyanobacteria in water bodies makes them good candidates as bioindicators for heavy metals. In this paper, a novel bioindicator using naturally occurring carotenoids for heavy metals detection is reported. The content of carotenoids in cyanobacteria decreased in the presence of heavy metals, with the measurement carried out using spectrophotometer ($\lambda = 440$ nm). From the experiment, the cyanobacteria from the lag phase and stationary phase were suitable to be used as the bioindicator, while the detection ranges of Cu and Pb were within 0.01 mg/L to 10.00 mg/L. The lowest detection limits for both Cu and Pb were 0.01 mg/L respectively.

Keywords—Heavy metals indicator, *Anabaena cylindrica*, Carotenoids

I. Introduction

Heavy metals are released into environment with an alarming rate due to anthropogenic activities. The widespread of heavy metals, especially in aquatic environment has threatened the survival of many organisms.

Biosensors can be powerful analytical tools to detect the presence of heavy metals in water without using expensive and highly expert-dependent analytical instruments, e.g. atomic absorption spectrometer. The wide spread of aquatic microorganisms, e.g. algae and cyanobacteria have been developed as bioreporters for biosensors [1-3]. Naturally available microorganisms have the advantages of showing the real physiological impact of heavy metals to the particular environment [4, 5].

By far, the study on cyanobacteria for biosensors' application were mainly focused on the photosynthetic pathways of these microorganisms, where the interference of the bioenergetics process by heavy metals could be measured through the changes of oxygen concentration and chlorophyll fluorescence emission [6]. The study using other pigments, such as carotenoids and phycocyanin are lacking.

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II. Methodology

A. *Cyanobacteria Culture and Determination of Growth Phases*

Jaworski's Medium (JM) was prepared based on the nutrients as recommended by Culture Collection of Algae and Protozoa, CCAP, UK [7]. The cyanobacteria *Anabaena cylindrica* was cultured in room temperature, with light to dark ratio maintained at 16 hours to 8 hours. The illumination was provided by white fluorescence light. Aeration was carried out with electric orbital shaker (Green S Seriker II, Vision) at 120 rpm to avoid clumping of cells. The culture was maintained for 16 days before subculture with new medium. All the glassware and disposables used for culturing were autoclaved. All the culture work was conducted in aseptic condition.

The growth curve of *A. cylindrica* was identified using cell counting method [8] using haemocytometer (Marienfeld-Superior, Neubauer) and light microscope (Eclipse E-100 LED, Nikon).

B. *Determination of Optimum Absorbance Wavelength*

A volume of 2 mL *A. cylindrica* cells in suspension was transferred into a cuvette. OD of the cells was measured using UV/Vis spectrophotometer (Spectronic Genesys 10UV, Thermo Scientific) within a range of $\lambda = 400$ nm – 800 nm. The highest absorbance wavelength in between 400 – 500 nm was selected to be used throughout the experiment.

C. *The Immobilization of Cells*

Immobilization was carried out using 0.5% (w/v) of agarose. The agarose powder was obtained from Fisher Scientific, Malaysia. Agarose solution was prepared by adding 0.5 g of agarose powder into 50 mL of distilled water. Then, 0.5 mL of cells in suspension was mixed with 0.5 mL of agarose solution in a cuvette at 45°C. The mixture was left in room temperature to solidify on a clear side of the cuvette. Cuvettes with the immobilized cell were stored in room temperature before the exposure to heavy metals.

D. *Exposure to Cu and Pb*

Different concentrations of Cu and Pb solutions were prepared using $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{Pb}(\text{NO}_3)_2$ provided by System, Malaysia. The immobilized cells were exposed to

0.01 mg/L, 0.10 mg/L, 1.00 mg/L, and 10.00 mg/L of heavy metals, with each cuvette used for single exposure. The change of OD was measured at 0 hour, 1 hour, and 24 hours after the exposure. The percentage of change in absorbance was calculated using (1). The exposure tests to Cu and Pb were carried out for the cells obtained from lag phase, exponential phase, and stationary phase.

$$\% \text{ of change} = A_1 - A_0 / A_0 \quad (1)$$

Where,

A_0 represents absorbance at time 0 hour

A_1 represents absorbance at a specific time

III. Results and Discussion

A. Determination of Growth Phases and Optimum Absorbance Wavelength

The growth phases of *A. cylindrica* were determined by cell count for 16 days, with the results shown in Fig. 1. The results showed a short lag phase for about 3 days after the subculture, followed by exponential phase from day 3 to day 10, while stationary phase followed from day 10 to day 16. The presence of three phases are in agreement with the results obtained by Wong et al. [8].

The result of scanning for the optimum absorbance wavelength is shown in Fig. 2. The peak at $\lambda = 440$ nm showed the closest absorbance with carotenoids as reported by Wong and Choong [9] and Ortiz et al. [10], thus $\lambda = 440$ nm was selected as optimum wavelength in this experiment.

B. Exposure to Cu and Pb

The exposure of *A. cylindrica* to heavy metals predominantly lowered the absorbance at $\lambda = 440$ nm for both Cu and Pb. Significant reduction in absorbance could only be noticed at 24 hours of exposure to heavy metal, for all the cells from three phases of growth. Thus, the exposure time of the cells to heavy metals was set at 24 hours. The change in absorbance (%) of *A. cylindrica* from lag phase exposed to Pb for 24 hours is shown in Fig. 3.

Carotenoids are photosynthetic pigments with the colour of yellow to orange, which can be widely found in photosynthetic organisms. β -carotene and xanthophyll are major types of carotenoids in cyanobacteria [11, 12]. *Anabaena* species have been determined containing high concentration of β -carotene [13]. Carotenoids have two main functions. First as additional light harvesting pigments and secondly, to assist chlorophyll a in light harvesting process and protects photosynthetic organisms from photo-oxidative damage through photo-protection [14, 15]. The presence of heavy metals increases the oxidative stress to cyanobacteria while causes changes in morphology and pigment composition, while damages essential enzymes and DNA [16, 17].

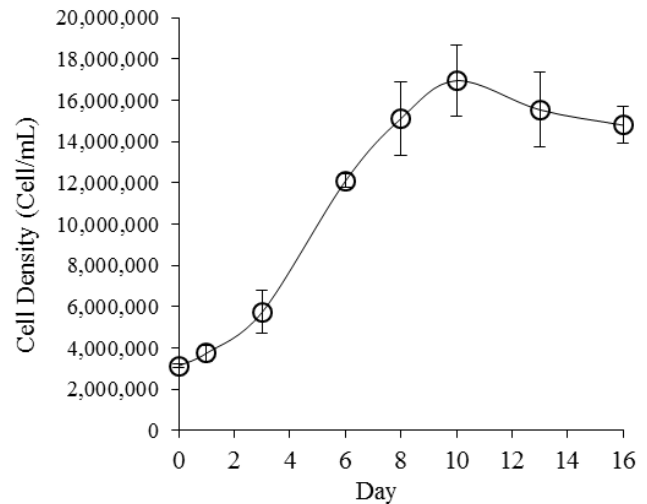


Figure 1. Growth curve of *A. cylindrica*

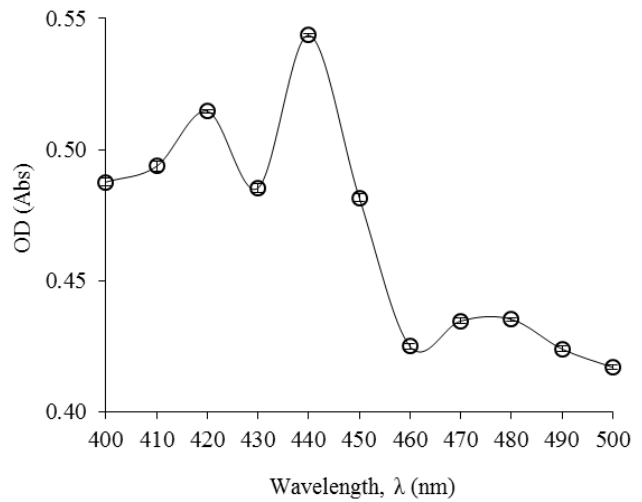


Figure 2. Optimum absorbance at $\lambda = 440$ nm

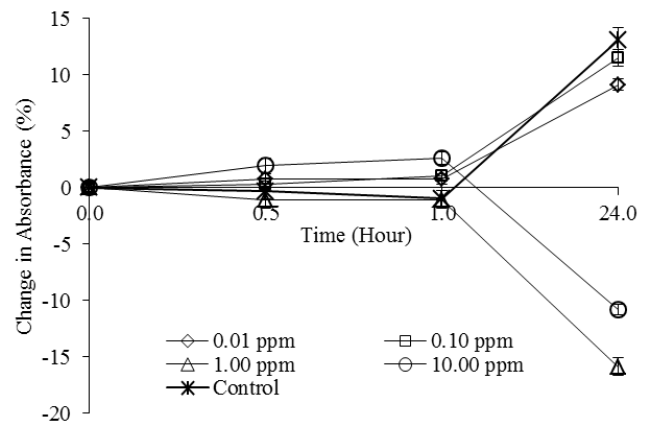


Figure 3. Exposure to Pb for 24 hours

The changes of absorbance in the presence of Cu and Pb for 24 hours were shown in Fig. 4, with blank (distilled water) was used as baseline. The results showed that the exposure to heavy metals caused the decrease in absorbance for the cells obtained from lag phase and exponential phase. The carotenoids content in *A. cylindrica* decreased as the heavy metals' concentration increased. The trend showed the carotenoids were utilized by the cells to mediate the damage by heavy metals. The result however was different compared to the works done by Wong and Choong [9] on *Daucus carota* and Chakilam [18] on cyanobacteria *Anabaena oryzae* and *Tolypothrix tenuis*, where the increase of carotenoids content in the presence of heavy metals were reported. The inconsistency might due to the difference in the microorganisms used. According to the experiment conducted by Rai et al. [19], the amount of carotenoids, chlorophyll, and phycocyanin in *Nostoc muscorum* were significantly reduced after 15 days of exposure to low concentration of heavy metals. Giotta et al. [20] reported the degradation of photosynthetic pigments of another species of cyanobacteria *Rhodobacter sphaeroides* in the presence of heavy metals as well.

The analysis on the best fitting linear detection ranges showed high correlation between the decrease of carotenoids content and the increase of heavy metals concentration. With the high R² value, the lag phase and the stationary phase of the bacteria could be used to serve as bioindicator for quantitative detection of Cu and Pb. The lowest detection limits for both Cu and Pb tested by far were 0.01 mg/L respectively.

TABLE 1. The possible linear detection ranges of the bioindicator

Heavy metal	Growth Phase	Linear Detection Range (mg/L)	Linear Equation	R ²
Cu	Lag	0.01 – 1.00	$y = -27.35x - 1.3953$	0.9740
	Exponential	0.01 – 10.00	$y = -2.7332x - 7.5655$	0.7938
	Stationary	0.01 – 1.00	$y = -19.706x + 2.1371$	0.9881
Pb	Lag	0.01 – 10.00	$y = -1.9743x - 2.8923$	0.9575
	Exponential	0.10 – 10.00	$y = -0.8638x - 6.9218$	0.8881
	Stationary	0.01 – 10.00	$y = -1.1038x - 0.9331$	0.9684

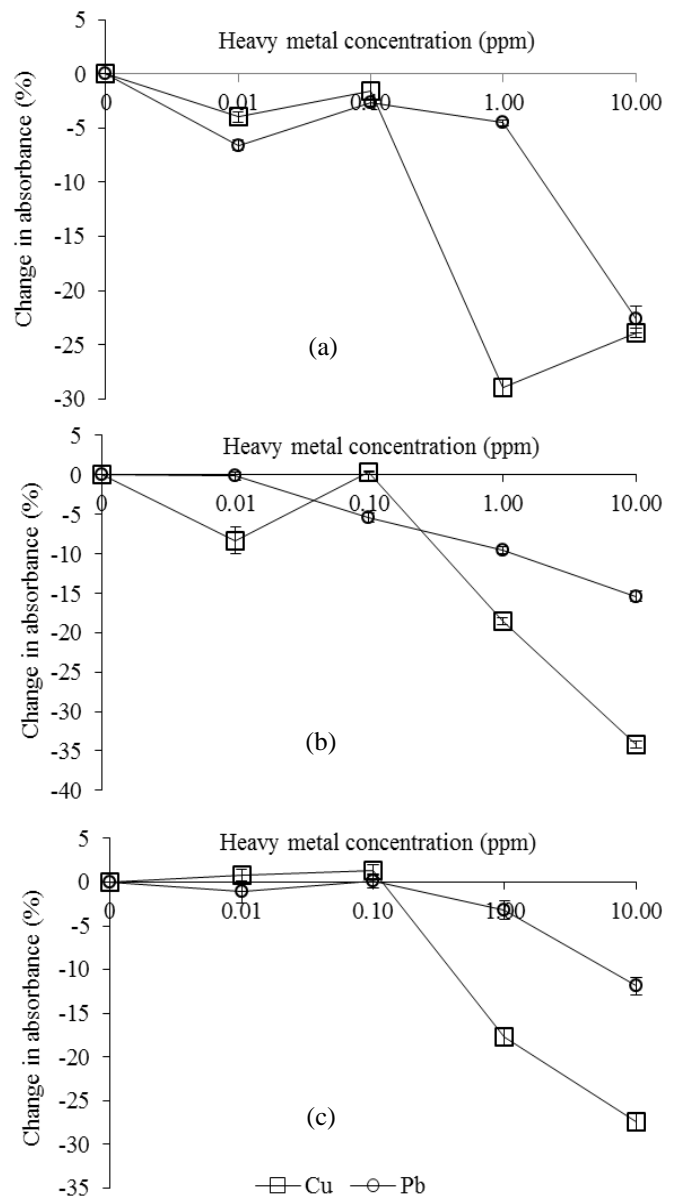


Figure 4. The absorbance changes after 24 hours of exposure to various concentrations of heavy metals for three growth phases of cells: (a) lag phase, (b) exponential phase, and (c) stationary phase.

IV. Conclusion

A novel bioindicator for heavy metals detection has been found. The changes of naturally occurring carotenoids content in cyanobacteria *A. cylindrica* was utilized, which the detection of carotenoids was conducted by measuring the absorbance with $\lambda = 440$ nm. With the exposure time of 24 hours and good correlation between the responses and the concentration of heavy metals, the bioindicator has the potential to be developed into biosensor for the quantitative analysis of heavy metals.

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