Fluorometric Response of Cyanobacteria to the Combination of Heavy Metals

Ling Shing Wong, Yook Heng Lee, and Salmijah Surif

Abstract—Polluted environmental samples always contain more than one kind of heavy metals. Therefore, to develop a biosensor for environmental application, the study of the response of biological elements to combination of heavy metals is crucial. In this study, the response of cyanobacteria Anabaena torulosa towards combination of heavy metals was determined. The response was captured by the fluorescence emission at 648 nm with the excitation at 526 nm, corresponding to the response of chlorophyll a in the cyanobacteria. The results showed the combined heavy metals affected the chlorophyll a agonistically, which the toxicity effect of the combined toxicity was less compared to the individual heavy metal. However, the increase in fluorescence caused by the combined toxicity effect, which was similar to the toxicity effect of the single heavy metal, enabled the chlorophyll a fluorescence to be used as the reporter group for qualitative analysis of heavy metals in environmental samples.

Keywords—About key words, separated by commas.

I. INTRODUCTION

Environmental pollution by heavy metals is a worldwide phenomenon. Anthropogenic activity is one of the major culprits to the release of heavy metals to the environment. To date, many approaches, e.g. analytical chemistry approaches and biological approaches have been developed to detect the presence of heavy metals.

In biological approaches, many cells, e.g. bacteria, algae, cyanobacteria, have been used as the bioindicators or biological reporter groups in biosensors [1]. These cells reflect the real physio-chemical toxicity of pollutants to the living organisms. As one of the smallest living entities, these cells can produce distinct responses towards certain analytes, with high sensitivity and rapid responses [2-4].

Chlorophyll fluorescence in photosynthetic cells has been selected as one of the indicators to detect the presence of heavy metals in several biosensors designs [5, 6]. The inhibition of heavy metals on electron transport chain in photosynthesis leads to the increase of chlorophyll fluorescence emission, which the detection can be accomplished using fluorometer. The biosensor designed by far, however, mostly based on the single toxicity test on individual heavy metals. The responses of the cells towards combination of heavy metals were not much reported.

In this study, the fluorescence response of cyanobacteria A. torulosa to the exposure of heavy metals was determined. In line with the development of biosensor, the exposure time of the cell was limited to 30 minutes. This study used the biosensor design as described by Wong et. al. [7].

II. METHODOLOGY

A. Chemicals and Glassware

Bold’s Basal Medium was prepared for the culture of cyanobacteria A. torulosa as recommended by Chia et al. [8] and Wong et. al. [5]. Heavy metals copper nitrate Cu(NO₃)², lead nitrate Pb(NO₃)², and cadmium nitrate Cd(NO₃)² were obtained from Merck, Germany. All solutions were prepared in deionized water. The glassware and medium required for culturing purposes were autoclaved at 121°C for 15 minutes. Polymer pHEMA was purchased from Sigma, United Kingdom.

B. Cell Culture and Immobilization

The cyanobacteria A. torulosa was cultured and immobilized as described by Wong et al. [5]. The immobilization was carried out with the cells from day-7 culture, using cellulose membrane as the supporting matrix. The immobilized cells were air-dried for 24 hours in room temperature. The density of cells was estimated 2.2 x 10⁶ cells / cm².

C. Heavy Metals Exposure

The fluorometric responses were captured using emission and excitation wavelengths of 526 nm and 648 nm respectively. The immobilized cells were exposed to single heavy metal Cu, Pb, and Cd, as well as the combination of Cu + Pb, Cu + Cd, and Cu + Cd. The immobilized cells were activated with distilled water before the exposure to heavy metals for 30 minutes. The change in fluorescence was calculated using Equation (1) below:

\[
\text{Change in fluorescence} = (E₀ - E₁) \times 100% / E₀
\]

Where:

\(E₀\) = Fluorescence intensity before the exposure

\(E₁\) = Fluorescence intensity after the exposure (30 minutes)

Combined heavy metals were prepared by mixing the heavy metals in 1:1 ratio (v/v). For example, the combination of Cd and Pb was prepared by mixing a volume of Cd solution (x mg/L) with a volume of Pb solution (x mg/L) to produce 2 a volume of Cd + Pb solution (x mg/L).

All the exposure tests were conducted under room condition, pH = 7, with n = 3.

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D. Calculation of Toxicity Unit (TU)

Toxicity unit was calculated to identify the antagonistic, synergistic, and additive effects for toxicity of heavy metals, as demonstrated by Backhaus et al. [9] and Kim et al. [10]. Toxicity Unit can be calculated using Equation 2 below:

\[ TU = \frac{c}{EC_{50}} \]  

Where,

- \( TU \) = Toxicity unit of a specific substance
- \( c \) = Effective concentration of the specific substance to produce a certain level of reaction, e.g. 5% of increase in fluorescence
- \( EC_{50} \) = Effective concentration of a specific substance to produce 50% of reaction

Value \( TU = 1 \) is equivalent to the concentration of specific, e.g. heavy metals that produce 50% of reaction. The synergistic, antagonistic, and additive effects can be determined by the Equation 3, Equation 4, and Equation 5 respectively as shown below:

\[ \Sigma TU_{A+B} > TU_A + TU_B \text{(Synergistic)} \]  
\[ \Sigma TU_{A+B} < TU_A + TU_B \text{(Antagonistic)} \]  
\[ \Sigma TU_{A+B} = TU_A + TU_B \text{(Additive)} \]

Where,

- A = Substance A
- B = Substance B

III. RESULTS AND DISCUSSION

A. Cell Culture and Immobilization

Cyanobacteria A. torulosa is originated from Nostocaceae family [11], with the average cell grow to a size of 6 – 10 µm in diameter. The cyanobacteria tend to form filamentous colonies and can be easily entrapped by polymer matrix. Cyanobacteria are fluorometric organisms as the cells contain chlorophyll \( a \) and undergo photosynthesis [12, 13]. When electrons in chlorophyll \( a \) receive photon energy, the electrons which are excited will release the energy through a few channels, in order for them to resume to their ground state.

Fluorescence emission is one of the channels where the energy absorbed is released through the release of a light photon [14]. According to Tortora et al. [15], microbes are most sensitive to external stimulus at the exponential growth phase. A. torulosa entered the exponential growth phase at day-7 of the culture. The emission and excitation wavelengths of at 526 nm and 648 nm were determined experimentally.

B. Heavy Metals Exposure

The presence of heavy metals inhibits photosynthesis thus increase the energy diffusion through fluorescence emission. Although Cu is required by plants as micronutrient, Prasad et al. [16] reported that Cu affected the degradation of pigments and photosynthesis rate of plants at 25 \( \mu \)g/L. Vavilin et al. [17] showed that the presence of Cu inhibited photosynthesis at quinone acceptor (\( Q_B \)) in electron transport chain in cyanobacteria. Pb is a non-beneficial heavy metal that inhibits photosynthesis by the binding to the oxidation and reduction site of PSII [18, 19]. Cd is another type of heavy metal which is able to inhibit photosynthesis by the binding to the donor site in PSII (Pagliano et al. 2006). Faller et al. [20] and Leborans and Novillo [21] reported that Cd competed with Ca and Zn for the binding site in PSII. Thus, in the presence of Cu, Pb, and Cd, the increase of fluorescence emission due to the inhibition of photosynthesis was anticipated.

Fig. 1, Fig. 2, Fig. 3, and Fig. 4 show the change of fluorescence emission (%) in the presence of different concentrations of heavy metals. The fluorescence intensity increased corresponding to the increase of heavy metals until a plateau, where the response was limited by the saturation of binding sites and the rate of the diffusion of the heavy metals through the cells.
C. Calculation of TU

The calculation of TU was carried out as recommended by Backhaus et al. [9] and Kim et al. [10]. The results showed the TU values of the combined heavy metals were lower than the sum of the TU for individual heavy metal (Tab. 1).

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>EC\textsubscript{50} (µg/L)</th>
<th>ΣTU\textsubscript{A+B}</th>
<th>ΣTU</th>
<th>Combined toxicity effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>6.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>4.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>2.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu + Pb</td>
<td>5.5</td>
<td>12.5</td>
<td>19.76</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>Cu + Cd</td>
<td>5.5</td>
<td>18.18</td>
<td>30.22</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>Pb + Cd</td>
<td>5.5</td>
<td>1.81</td>
<td>2.77</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>Cu + Pb + Cd</td>
<td>6</td>
<td>5</td>
<td>8.36</td>
<td>Antagonistic</td>
</tr>
</tbody>
</table>

The information of cyanobacteria response to combination of heavy metals is important as most of the polluted samples from environment contain more than one type of toxicants (Cooper et al. 2009). Although many of the test on the combination toxicity showed either antagonistic, synergistic, and addition effects, some other unpredictable possibilities were presence as well [22-25].

IV. CONCLUSION

In conclusion, the fluorometric response of \textit{A. torulosa} towards the combination of Cu, Cd, and Pb resulted in antagonistic effects. The results showed by measuring the fluorescence of chlorophyll \textit{a} in the cyanobacteria, the presence of heavy metals in a water sample could be detected. Although the co-presence of heavy metals in a specific water sample might lower the response, the quantitative analysis could still be conducted. Thus, the chlorophyll \textit{a} fluorescence could be used as a measuring parameter to detect the presence of single and combination of heavy metals.

REFERENCES