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Fluorometric Response of Photosynthetic Microorganism Consortium as Potential Bioindicator for Heavy Metals Detection in Water

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Abstract

This paper reports the fluorometric responses of photosynthetic microorganism consortium in the detection of heavy metals (Cu and Cd). The consortium was collected from natural water body, and then exposed to different concentration of heavy metals. The response was measured based on the fluorescence signal emitted by the consortium before and after the exposure to heavy metals, with excitation and emission wavelengths set at 526 nm and 648 nm respectively. Cell suspension with optical density (OD) of 0.75 at λ = 700 nm was found to produce best response. The ability of the consortium to respond to both heavy metals within 0.01 – 10.00 mg/L was confirmed. Thus, photosynthetic microorganism consortium could be a good natural candidate as heavy metals bioindicator.

Keywords: Photosynthetic Microbes; consortium; fluorometric response; heavy metals; bioindicator

1. Introduction

Heavy metals pollution has become a serious environmental issue, affecting not only the flora and fauna at the surrounding area, but threatening human life as well through food chain and drinking water. Cu and Cd are two heavy metal pollutants which are commonly found in wastewater discharged from mining, electroplating, smeltingand pigmenting industries (Li et al., 2004). Due to the overwhelming pollution issues, sensitive scientific tools have been developed for rapid detection of pollutants. However, most of these tools, e.g. atomic absorption spectrometry and inductively coupled

plasma-mass spectrometer require high operational cost and maintenance. Hence, whole cell algae bioindicator can be used as alternative to screen for the presence of heavy metal pollutants.

The search for new cost effective methods for the detection of heavy metals has been focused on the usage of photosynthetic organisms (Bilitewski et al., 2004). Microalgae are generally low cost, easy to cultivate, present abundantly in aquatic ecology, and they are very sensitive to environmental changes (Torres et al., 2000; Chouteau et al., 2004). These microalgae contain chlorophylls and have the ability to produce oxygen (Beams & Kessel, 1977). The energy received from the sun is diffused through the conversion to heat, channeled to photosynthesis process, and some of the energy are released in the form of fluorescence emission. The presence of heavy metals in microalgae will inhibit the photosynthesis process and causes change in the fluorescence emission (Wong et al., 2013). Hence, fluorescence emission is a suitable parameter to be used in the detection of heavy metals. Up to now, several bioindicators have been successfully constructed using microbes, e.g. yeast (Mastura et al., 2013), algae (Singh et al., 2012) and cyanobacteria (Wong et al., 2013).

To date, the research on the detection of heavy metals using cell-based bioindicator are focused on the specific species of lab-based cultures, such as microalgae Spirogyra sp. (Wong & Kiew, 2016) cyanobacteria *Anabaena cylindrica* (Paul et al., 2016) or plant cell *Daucus carota* (Wong et al., 2015). However, the full potential of using photosynthetic microorganisms, especially in the form of consortium from natural environment has not been explored (Hannon et al., 2010). As the responses of pure species of photosynthetic microbes are well studied, the responses of the consortium of photosynthetic microbes remained unknown. Therefore in this paper, the fluorometric response of chlorophyll in the photosynthetic microorganism consortium to the presence of heavy metals is reported. The outcome of this research might be useful in utilizing the photosynthetic microorganism consortium for the construction of biosensor.

2. Materials and Methods

2.1 Culture Medium and Chemicals

Bold Basic stock medium was purchased from Sigma-Aldrich, Malaysia. The stock medium was diluted 50x with deionized water for culturing work. Jaworski Medium was prepared based on the composition provided by Culture Collection of Algae and Protozoa (CCAP), United Kingdom. Heavy metals were obtained from Merck, Malaysia.

2.2 Microalgae culture and Cell Identification

Algae samples were collected from water sources from Biru Kundang Lake, Selangor (3.251160, 101.526124). The sample was cultured in combination of Bold Basic Medium and Jawoski Medium (ratio 1:1) to provide the nutrients required by the microalgae. Aeration was carried out daily by manual shaking. Light and dark conditions were at 16 hours and 8 hours respectively. The genus of the algae and cyanobacteria were identified through light microscope (Eclipse E-100 LED, Nikon).

2.3 Standardization of the Number of Cell

The standardization of algae consortium cells were done by spectrophotometer (Gene Quant 100, GE) with OD_{700nm} = 0.75. The consortium with OD_{700nm} = 0.75 contained optimum density of cells for the fluorescence study, which was experimentally determined. The intensity of fluorescence emission were captured using emission and excitation wavelengths sets at 526 nm and 648 nm respectively using spectrofluorometer (Glomax Multi Jr., Promega).

2.4 Heavy Metals Exposure

Cd solutions were added to 2 mL of consortium culture in four sided clear cuvettes to make the final concentrations of Cd in the algae consortium culture 0.01 mg/L, 0.05 mg/L, 0.10 mg/L, 0.50 mg/L, 1.00 mg/L, 5.00 mg/L, and 10.00 mg/L respectively. The intensity of the fluorescence emission was measured before the exposure to Cd, and then measured again after 30 minutes, 60 minutes, 120 minutes, 240 minutes, 360 minutes, and 480 minutes of exposure. The experiment was then repeated with Cu replacing Cd. All exposure tests were conducted in triplicates. The percentage of changes of the intensity of fluorescence emission was calculated using an equation below:

Increase in fluorescence emission (%)
$= (f_1 - f_0) \times 100\% / (f_0)$;

Where :

f_0 = fluorescence emission before the exposure;

f_1 = fluorescence emission after the exposure

3. Results and Discussion

3.1 Microalgae Identification

Co-culture or consortium had the advantages in certain applications which were impossible to be done by an individual strain or species (Brenner et al., 2008). Potential of photosynthetic microorganism consortium as the bio-indicator have been evaluated and it showed that they were able to detected present of heavy metals through the production of fluorescence emission. The consortium contained *Chlorella* sp., *Haematococcus* sp., cyanobacteria and diatoms.

3.2 *Effects of copper and cadmium on photosynthetic microorganism*

The density of cell can be determined by the reading of OD at 700 nm, which is the wavelength for absorption of chlorophyll a (Desikachary, 1959). The correlation between the optical densities of consortium and fluorescence emission is portrayed in Figure 1. OD has been used to determine the number of fluorescence bacteria (Nawaz et al., 2011), and cyanobacteria (Wong et al., 2013). From the experiment, fluorescence increased from 0.10 A – 0.60 A. However, the intensity started to decreased when the increment of consortium culture exceeded 0.75 A, due to the reabsorption of fluorescence emitted by nearby cells (Védrine et al., 2003). Based on the result, the consortium culture at OD_{700nm} = 0.75 yielded the highest fluorescence intensity, thus had been used for the exposure tests.

The fluorometric response of consortium to seven different concentrations of Cd is shown in Figure 2. Cd at 0.05 mg/L yielded the highest fluorescence intensity compared to other Cd concentrations. However, low fluorescence intensity was observed at concentration of 5 mg/L and 10 mg/L. Lower fluorescence intensity yielded at the higher concentrations of Cd might due to disturbance of metabolic activities of the photosynthetic microbes. In photosynthetic organisms, Cd affected metabolic activities such as photosynthesis in both PS I and PS II (Atal et al., 1991). The effect might due to the difference way of reaction of microbes reacted to the heavy metal (Gadd *et al.,* 2007; Gadd et al., 2009).

The responses of the cells to Cu is shown in Figure 3. The concentration of Cu at 0.05 mg2L and 0.50 mg2L yielded the highest fluorescence intensity. Cu is an essential element that is required by the metabolic processes of algae (Campanella et al., 2001).

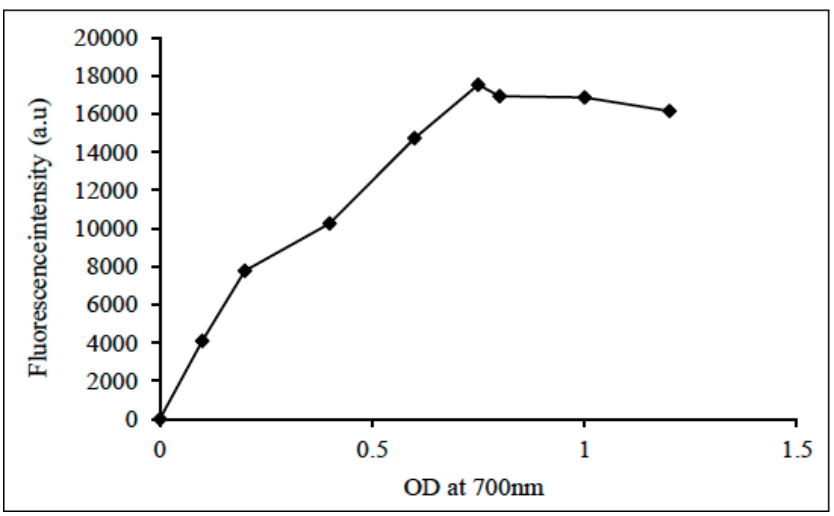

Figure 1. The correlation between OD and fluorescence intensity

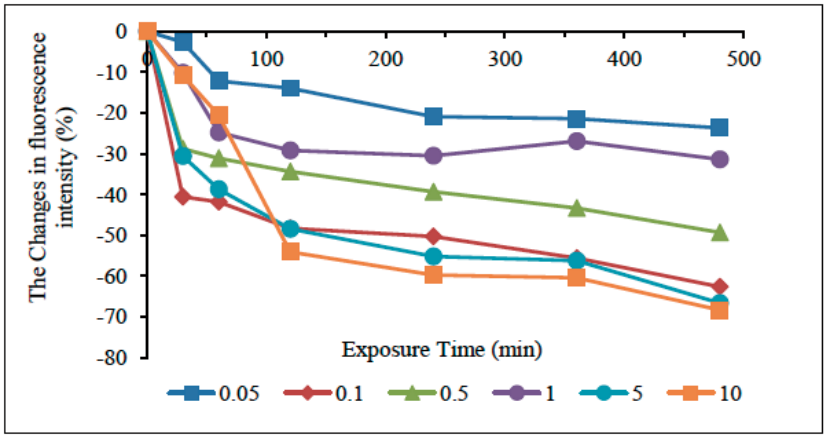

Figure 2. Exposure of consortium to different concentration of Cd. The changes of fluorescence intensity were significant ($p < 0.05$) compared to negative control.

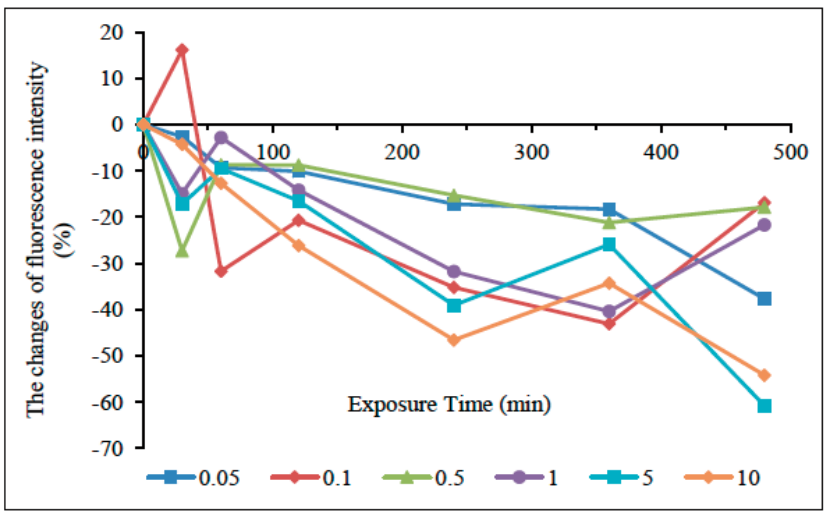

Figure 3. Exposure of consortium to different concentration of Cu. The changes of fluorescence intensity were significant ($p < 0.05$) compared to negative control.

However, the metal would exhibit toxicity effect in high concentration (Pandard et al., 1993).

4. Conclusion

The result from this study confirmed that the algae consortium responded to the presence of heavy metals through fluorescence emission. Although the test showed different responses, the consortium produced detectable emission signal to indicate the disturbance by Cu and Cd to the consortium culture, which enabled qualitative detection of the heavy metals. This study showed the consortium could detect the presence of heavy metals even at concentration as low as 0.05 mg/L within 30 minutes of exposure.

5. Acknowledgement

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