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Response of *Chlorella vulgaris* as Whole-cell Bioindicator for Atrazine and 2, 4-Dichlorophenoxyacetic Acid Detection

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***Abstract*—Microalgae are widespread organisms which can be used as bioindicator for pesticides in water. The inhibition of photosynthesis by pesticides, which leads to the change in fluorescence emission can be used as the parameter of detection. In this study, the responses of microalgae *Chlorella vulgaris* to two pesticides- atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D) were reported. For the optimized responses, *C. vulgaris* from day-3 culture with approximate 3.5 x 10^6 cells was immobilized with 0.5 % of agarose, which later exposed to different concentrations of atrazine and 2,4-D for 15 minutes respectively. The results showed *C. vulgaris* responded to both pesticides from the range of 0.001 mg/L – 10.000 mg/L. The results confirmed the potential of *C. vulgaris* as bioindicator for both pesticides.**

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

I. Introduction

Microalgae are different collections of prokaryotic and eukaryotic photosynthetic microorganisms, regularly found in freshwater and marine environment. There is a high percentage of photosynthetic carbon digestion accomplished by algae [1].

The broad usage of herbicides in agricultural field has caused the deterioration of water quality. The presence of herbicides inhibits photosystem II (PSII) activities by targeting quinone binding site, stopping photosynthesis [2]. Thus photosynthetic inhibition is the indicator which imitates toxic effect of pollutants.

Typical methods and approaches such as high-performance liquid chromatography (HPLC) and mass chromatography (MS) require skilled technicians and expensive equipment. Bioindicator is the alternative method have been identified to be the most cost effective with high sensitivity. Bioindicator is defined as biological component that that can be used to analyze and monitor the health of ecosystem. One of the most commonly available bioindicator is naturally occurring green algae.

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Chlorella vulgaris is unicellular organism and it is one of the most popular deliberated and researched algae species. It is one of the fastest growing microalgae which is available in wide range of aquatic environment [3, 4]. It often used as bioindicator because of its ability to respond quickly and high sensitivity towards toxicants which are mostly herbicides [5]. This paper reports the potential of *C. vulgaris* as a bioindicator to two types of herbicides through the determination of changes in fluorometric emission.

II. Methodology

A. Cell culture and Immobilization

Bold Basal Medium was used in *C. vulgaris* culture. 10 mL of culture was transferred into 100 mL of fresh medium, and was kept from 15 days before the next sub-culture. The culture was maintained 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 SSeriker II, Vision) at 120 rpm to avoid clumping of cells. All the glassware and disposables used for culturing were autoclaved. All the culture work was conducted in aseptic condition.

For immobilization of cells, a total of 0.5 mL of agarose solution (1%) was mixed with 0.5 mL of *C. vulgaris* culture in a plastic cuvette. The mixture was left to dry in room temperature for 30 minutes.

B. The Exposure of Cells to Pesticides

Atrazine and 2,4-D stock solution were prepared, with different concentrations of 0.001 mg/L, 0.010 mg/L, 0.100 mg/L, 1.000 mg/L, and 10.000 mg/L obtained through serial dilution.

The optimization through experiments determined the *C. vulgaris* cells from day-3 culture with time of exposure set at 15 minutes produced the best responses. The estimated number of cells was 3.5 x 10^6 per cuvette.

Then, 1 mL of atrazine with concentrations of 0.001 mg/L, 0.010 mg/L, 0.100 mg/L, and 1.000 mg/L were added to the immobilized cells respectively. The broad spectrum fluorescence intensity before the exposure and 15 minutes after the exposure were measured using a fluorometer (GeneQuant 1300, GE) with emission detection at 300 – 700 nm. The change in fluorescence intensity was calculated in

percentage (%). The change in fluorescence intensity was then compared to the negative control (the immobilized cells exposed to distilled water). The exposure tests were then repeated with 2,4-D replacing atrazine. All exposure tests were conducted in triplicates.

The percentage of change in fluorescence was calculated using Equation (1).

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

Where,
A_0 = % of change in fluorescence intensity at 0-minute
A_1 = % of change in fluorescence intensity at 15-minute

III. Results and Discussion

A. *Cell Culture and Immobilization*

C. vulgaris cells were cultured in BBM medium which was an inorganic salts medium that contained essential macronutrients and micronutrients for optimum growth of freshwater algae [6]. When *C. vulgaris* cells entered a new habitat and faced different nutritional conditions they experiment with what was known as lag phase. A metabolic re-adaptation of the cell caused it to develop in the new environment [7].

Once *C. vulgaris* cells became adapted to the new environmental condition, they started to divide and grow exponentially by increasing in the number of cells as reported by Ruiz-Marin, Mendoza-Espinosa & Stephenson [8]. It was an occurrence known as exponential phase or log phase, which observed from day-2 to day-7 respectively. During this exponential phase, the cell number increases significantly and more sensitive to the change in environment [9]. Thus, the cells from exponential phase was used in this experiment.

Agarose was used as an immobilizing agent in this experiment as it was a strong gelling agent, easily available, low in cost, and it did not require any additional substances to prepare as compared with other elements that normally needed for immobilization [10].

B. *The Exposure of Cells to Pesticides*

Figure 1 shows the response of *C. vulgaris* after the exposure to different concentrations of atrazine for 15 minutes. The *C. vulgaris*, cells which had been exposed to 0.001 mg/ L atrazine gave the lowest response of -6.43 %, while the highest response yielded was -36.10 % at 10 mg/L.

The response of *C. vulgaris* decreased as the concentration of atrazine increased. The obtained pattern of the graph was in agreement with El-Sheekh et al. [11] and Hersh & Crumpton [12] who demonstrated the effect of atrazine on photosynthesis, growth and protein synthesis in the unicellular green algae.

Figure 2 shows the response of *C. vulgaris* towards five different concentrations of 2,4-D. After comparison with a negative control, cells which had been exposed to 0.001 mg/L gave a response of +5.00 % and -4.21 % at 10 mg/L for 15 minutes of exposure.

Saygideger & Okkay [13] reported the effect of 2,4-D on growth and chlorophyll content of *C. vulgaris* and *Spirulina plantensis* with similar result. Studies by Pazmiño et al. [14] have suggested that synthesis of auxin could be disrupted due to higher concentration of 2,4-D, whereas carbon assimilation and photochemical reaction might be strengthened through low concentration of 2,4-D.

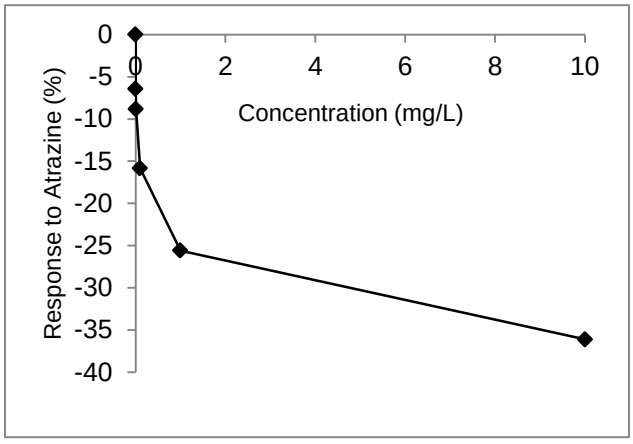

Figure 1. The response of *C. vulgaris* towards different concentrations of atrazine solution in 15 minutes of exposure.

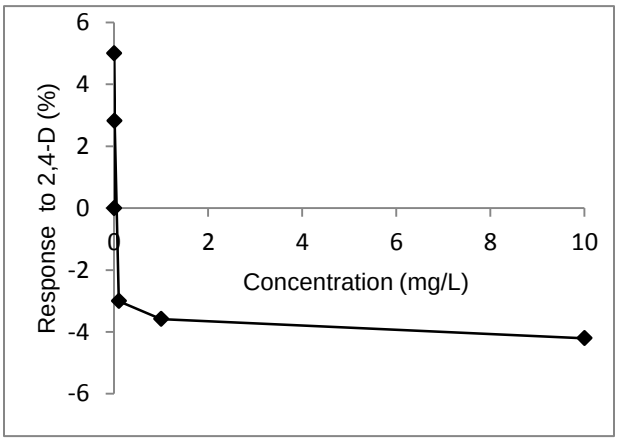

Figure 2. The response of *C. vulgaris* towards different concentrations of 2,4-D solution in 15 minutes of exposure.

Previous research done by Védrine et al. [15] on whole-cell biosensor using *C. vulgaris* for monitoring herbicides showed that the algae could response affectively in the presence of atrazine. In this study, an optimal pH of 7 was maintained in room temperature. While Wong et al. [16] reported the usage of photosynthetic microbes in 2,4-D detection at– culture pH at room temperature. These reports consolidate the potential of using the algae as bioindicator for the detection of both atrazine and 2,4-D.

IV. Conclusion

An experiment conducted to test the responses of *C. vulgaris* to two types of pesticides- atrazine and 2,4-D had been successfully conducted. The exposure of the pesticides shown the best responses obtained from the density of 7 x 10^6 cells/mL, with the cells from day-3 culture and the exposure time of 15 minutes. Both of the pesticides caused a decrease in the photosynthetic response of *C. vulgaris* as the concentration of the pesticides increased. The, response produced by *C. vulgaris* towards different concentrations of herbicides demonstrated it could be applied as a whole-cell bioindicator to detect the herbicides with fast responding detection, cheaper in cost and the fact that it is available in abundance.

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