Development of Herbicide Detection Method Using Microalgae as a Biosensor

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Introduction

The microalgae, *Chlorella vulgaris* has been reported to be a sensitive indicator for photosystem II inhibitors. Tests using the microalgae revealed that chlorophyll fluorescence greatly increases in the presence of photosystem II inhibitors in the media. Based on this study, we investigated the microalgae obtained locally i.e. *Chlorella vulgaris*, *Scenedesmus quadriqauda* and *Chlorococcum* spp and used them in toxicity evaluations of photosystem II inhibitors. The three species of microalgae were isolated, purified and cultured in the laboratory. Evaluations of the toxicity of the herbicides atrazine, simazine and diuron were carried out by chlorophyll fluorescence and measurement of the rate of oxygen produced during photosynthesis.

Materials and Methods

The microalgae *Chlorella vulgaris*, *Scenedesmus quadriqauda* and *Chlorococcum* spp were first isolated from the Hulu Pangsoon water reservoir and a pond in Universiti Putra Malaysia, respectively. The microalagae were purified and cultured in the laboratory under optimised conditions for growth. The cells were determined for chlorophyll content, cell density, growth rate, optimum light:dark cycles, temperature and light intensity. Once the parameters were optimised, culture of the microalgae was initiated. For measurement of photosynthesis, determination of chlorophyll fluorescence in free cells and rate of oxygen produced were carried out. Photosystem II inhibitors used were atrazine, simazine and diuron. The herbicides were added to the culture media and chlorophyll fluorescence and rate of oxygen production were determined. Free *Chlorella vulgaris* were then immobilized in alginate beads of 2 mm diameter. Conditions for the formation of the microlagae in spherical beads were optimized. Free and immobilized *Chlorella vulgaris* were subjected to herbicide toxicity evaluations by measuring the rate of oxygen released during photosynthesis.

Results and Discussion

Of the three microalgae isolated and cultured in the laboratory, *Chlorella vulgaris* exhibited the best growth rate, chlorophyll content/cell and chlorophyll fluorescence compared with *Scenedesmus quadriqauda* and *Chlorococcum* spp., respectively. *Chlorella vulgaris* was found to be more sensitive to the herbicides atrazine, simazine and diuron than the other microlage species. Chlorophyll fluorescence of *Chlorella vulgaris* in the presence of the herbicides were significantly increased and from a mathematical formula derived from the results obtained, it is possible to extrapolate and determine the minimum concentration of herbicide required to inhibit photosynthesis.

Due to technical problems associated with the free-living nature of *Chlorella vulgaris*, an attempt was made to immobilize the cells so that a much easier and rapid method can be used to determine the change in the rate of photosynthesis. Round spherical alginate beads of 2 mm diameter were formed with varying concentrations of *Chlorella vulgaris* trapped in the beads were produced. Since the cells were entrapped in beads, chlorophyll fluorescence could not be determined. Thus, the oxygen electrode was used to determine quantitatively the rate of oxygen released during photosynthesis. A comparison was carried out between free cells, a standard concentration of trapped cells and varying concentrations of trapped cells in alginate beads with respect to herbicide toxicity and rate of photosynthesis. The trapped cells all exhibited a lower rate of oxygen released compared with the free cells. For herbicide toxicity evaluations, the trapped cells also exhibited a lower rate of oxygen released than free cells and control cells.

Conclusions

Measurement of herbicide toxicity is most useful using free *Chlorella vulgaris* by chlorophyll fluorescence. It is not recommended to use immobilized cells for the determination of herbicide toxicity using the oxygen electrode.

Benefits from the study

A cheap and easy method for determining contamination of water by herbicide using free Chlorella vulgaris and chlorophyll fluorescence has been developed. The method for encapsulation of the microalgae *Chlorella vulgaris* in alginate beads has been optimized. The trapped cells have potential uses.

Literature cited in the text

None.

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