DEVELOPMENT OF HERBICIDE DETECTION METHOD IN WATER USING MICROALGAE AS A BIOSENSOR

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Introduction

The triazines and urea herbicides are known to inhibit electron transport in photosystem II of higher plants. The herbicides exert their effect by binding to specific proteins in the photosystem II reaction centres (Pfister et al. 1974). The inhibition of photosystem II was detected spectrofluorimetrically in which chlorophyll *a* exhibited different fluorescent properties in the presence of herbicides (Duysens and Sweers, 1963). Since the fresh water algae, *Chlorella vulgaris* is abundant in water bodies, we adopted the spectrofluorimetric method for the detection of herbicide binding to chlorophyll *a* in *Chlorella vulgaris.* It is hoped that the change in fluoroscent properties of chlorophyll *a* in *Chlorella vulgaris* may lead to it being used as a biological indicator of herbicide contamination in fresh water bodies.

Materials and Methods

Chlorella vulgaris was first isolated from a pond in Universiti Putra Malaysia. It was then cultured in liquid media at 25°C, 900-1000 lux light and 12:12h light dark cycle long term (James, 1978). Aliquots from the stock culture were transferred to Bold Basal media for short term culture under similar conditions and16:8h light-dark cycle. Fresh aliquots from the short-term cultures were added to Bold Basal media, exposed to 12:12h light-dark cycle and subjected to treatment before being assayed for herbicide inhibition of photosynthesis. Cell number and density, chlorophyll content, growth rate and the effect of the herbicides; atrazine, simazine and diuron on the fluorescence of chlorophyll *a* was monitored. The results obtained were analysed statistically.

Results and Discussion

Chlorella vulgaris was observed to be relatively easy to culture. It showed exponential growth between 5-21 days in culture after which the culture showed stationary growth phase. On average, it showed an increase of approximately 206250 cells per day. In line with the exponential growth rate, *Chlorella vulgaris* exhibited an almost linear increase (312500 cell per Liter culture) in cell density over 21 days in culture. The chlorophyll content also exhibited a similar rate; an 18-fold increase was recorded over 14 days (day 1, 0.09 ng/cell; day 14, 1.6 ng/cell). Since the intensity of fluorescence is directly associated with chlorophyll concentration, the relative fluorescence of the intact alga was also increased in tandem with the growth rate. Spectrofluorimetric studies of the alga at day 14 in culture was carried out in the presence of different herbicide concentrations (0.1-1000 uM in five steps). Generally, a gradual ascending curve was observed in increasing herbicide concentrations. Mathematical treatment was applied and a relationship between relative fluorescence of chlorophyll *a* and herbicide concentration was determined. An equation for the effect of each herbicide on the alga used was obtained. Applying the equation on the actual readings obtained in experiments revealed that the method of measuring relative fluorescence of intact alga in the presence of herbicides could detect a much lower herbicide concentration than that used in the study. The lower limit for atrazine was 0.04 uM, simazine 0.05 uM and diuron 0.09 uM; these values are below the lower limit used in the study. This method is capable of detecting the herbicides at a much lower concentration than the safety limit set by various authorities for safe drinking water. The limit for atrazine is 20 uM in Australia (Cooper, 1994), 0.35 uM in USA (Meisner et al. 1993) and 2 uM in Canada (Maguire and Tkacz, 1993).

Conclusions

Chlorella vulgaris was found to be suitable for use as a biological indicator for herbicide contamination in fresh water bodies. Spectrofluorimetric measurement of intact alga for the detection of atrazine, simazine and diuron in water was found to be sensitive and capable of detecting levels below 0.1 uM herbicide concentration.

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