UNIVERSITI PUTRA MALAYSIA

THE DEVELOPMENT OF PHOTOSYSTEM II - HERBICIDE DETECTION METHOD USING MICROALGAE AS A BIOSENSOR

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THE DEVELOPMENT OF PHOTOSYSTEM II - HERBICIDE DETECTION METHOD USING MICROALGAE AS A BIOSENSOR

By

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Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Science and Environmental Studies, Universiti Putra Malaysia

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Dedication

to Mak, Ayah, Kak Safinaz, Kamal and Adik,

to my beloved husband, Zuhaidi Mudrim,

to all my supportive friends

May Allah Bless All of You

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LIST OF ABBREVIATIONS

BBM	Bold Basal media
Chl	Chlorophyll
EPA	Environmental Protection Agency
GC	Gas chromatography
PSII	Photosystem II
Q	quinone
UPM	Universiti Putra Malaysia

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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BY

SHAKINAZ BT DESA

JUNE 1997

Chairman : Assoc. Prof. Dr. Nor Aripin bin Shamaan

Faculty : Science and Environmental Studies

Herbicides have been found to be significant pollutants of water bodies. In order to control and monitor the pollution, a convenient, sensitive and reliable method is needed. The main objective of this study is to develop a new method to measure the fluorescence yield of microalgae in order to detect photosystem II (PSII) specific herbicides in polluted water. The system was based on a simple mixing of microalgae with PSII specific herbicides. The measured signal was in

the form of fluorescence intensity. The study involved the screening of test organisms, determining the best condition of the culture and obtaining a standard curve for each of the PSII herbicides. Only three herbicides were studied; atrazine, simazine and diuron. *Chlorella vulgaris* was found as the most suitable test organism compared with *Chlorococcum sp.*, *Scenedesmus quadriqauda* and *Selenastrum sp.*. Nutrient replacement culturing method was selected as the best condition to grow *C. vulgaris*. A standard curve was determined for each of the herbicides. *C. vulgaris* was able to signal atrazine's concentration above 0.04µM according to the standard curve equation. Simazine can be signalled at above 0.05µM from its equation, while diuron can be signalled at above than 0.09µM. However, this basic study needs more information and improvement to be done in the future, so that its full potential can be realised.

Abstrak tesis diserahkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains.

PEMBENTUKAN KAEDAH MENGESAN RACUN RUMPAI FOTOSISTEM II MENGGUNAKAN MIKROALGA SEBAGAI BIOSENSOR

Oleh

SHAKINAZ BT DESA

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Racun rumpai merupakan salah satu bahan cemar utama di dalam badan air. Bagi mengawal dan mengesan pencemaran, kaedah yang mudah, sensitif dan dipercayai adalah diperlukan. Objektif utama kajian ialah untuk membentuk kaedah baru bagi mengukur floresen mikroalga dalam mengesan racun rumpai fotosistem II (FSII) yang spesifik di dalam air tercemar. Sistem ini berasaskan pencampuran mikroalga dan racun rumpai FSII spesifik. Isyarat diukur di dalam

bentuk keamatan floresen. Kajian ini melibatkan pemilihan organisma ujian, kajian pengkulturan terbaik dan mendapatkan keluk piawai bagi setiap racun rumpai ujian. Tiga racun rumpai yang dikaji; atrazin, simazin dan diuron. *Chlorella vulgaris* didapati merupakan organisma ujian yang paling sesuai berbanding *Chlorococcum sp.*, *Scenedesmus quadriqauda* dan *Selenastrum sp.*. Kaedah penggantian nutrien dipilih sebagai kaedah pengkulturan terbaik bagi menumbuhkan *C. vulgaris*. Satu keluk piawai diperolehi untuk setiap racun rumpai setiap satu. *C. vulgaris* berupaya mengesan kepekatan atrazin di atas 0.04μM mengikut persamaan keluk piawai. Simazin boleh dikesan pada kepekatan di atas 0.05μM daripada persamaannya. Manakala diuron dapat dikesan di atas 0.09μM. Bagaimanapun, kajian asas ini memerlukan lebih banyak maklumat dan pembaikan di masa hadapan bagi membolehkan penggunaan potensi sepenuhnya.

CHAPTER I

INTRODUCTION

Current methodologies for pesticide detection, particularly herbicides, are not ideally suited for large scale monitoring programme. These include gas chromatography (GC) and high performance liquid chromatography (HPLC). These techniques need large sample volumes, extensive extraction and clean up procedures for analyses. The chemical analysis of a water sample is expensive and very time-consuming. Thus, the number of analyzed samples is limited.

Other techniques include flow cytogenic analysis using whole cell clastogenicity (Biradar and Rayburn, 1995); photoacoustic spectroscopy on excised leaf (Fuks et al., 1992); leaf disc buoyancy (LDB) method (Saltzman and Heuer, 1985); oxygen electrod method (Saka and Chisaka, 1982); treatment of activated carbon, reverse osmosis and deionisation (Delavaud et al., 1992); measuring alleviation of phytotoxic injury (Yanase et al., 1990) and soil extraction method by enzyme immunoassay and gas chromatography (Del Valle and Nelson, 1994).

A good analytical method must be accurate, precise, sensitive and selective. Therefore, we need a method that is capable to measure low concentrations of herbicides, low cost, fast result, easy handling and able to monitor large scale programme. The purpose of this study is to develop a method that utilises natural phenomenon as an early warning system. The method should be able to detect herbicide at below the level of permissible concentration.

In this work, the basic principle is based on the measurement of changes in the yield of variable chlorophyll *a* - fluorescence of PSII. The method involves a test organism and a spectrofluorometer. Aquatic organisms are used for toxicology tests because they experience the changes in the aquatic environment.

The changes of yield of the *in vivo* chlorophyll *a* - fluorescence of photosystem II are correlated quantitatively with the concentration of herbicides. Therefore, it can be used to set up a low price monitoring system and to be a promising basis for a detection device. Other studies that share the same principle were done by Jansen et al. (1993), Yanase and Andoh (1992), Duke et al. (1991), Voss et al. (1984), Richard et al. (1983) and Bohme et al. (1981).

As microalgae grow in aquatic environment, they are exposed to environmental changes. These disturbance, however, are adapted by microalgae in various way. For example, some increase the cell density (Trifonova, 1993), produce spore or resting stage (Nixdorf and Hoeg, 1993) or by migrating (Vincent, 1990). However, there are microalgae that could not survive with the changes. But, these microalgae can serve as a simple plant cell model in the herbicidal sites test systems (Fedtke, 1993).

The inhibition of herbicide towards the photosynthetic electron transfer can be detected by measurement of chlorophyll fluorescence (Gleiter and Renger, 1993; Percival and Baker, 1991; Habash et al., 1985). Fluorescence is a reradiated light energy fraction which cannot be used to drive photosynthetic electron transfer. The inhibition results in an increased fluorescence intensity at constant excitation intensity (Schrotter et al., 1994).

There are three objectives in this study. Firstly, to select the best test organism. Secondly, to determine the selected test organism obligation and thirdly to obtain a relationship between the fluorescence yield and herbicide concentration.

CHAPTER II

LITERATURE REVIEW

Herbicide

Introduction

Herbicides are chemicals with the potential to kill and destroy plants (Cobb, 1992). Herbicide can inhibit photosynthesis by interfering with :

1. the reproduction, development, structure, and integrity of chloroplasts,
2. biosynthetic pathways that are involved in the production of output products such as starch, amino acids synthesis,
3. photochemical induction pathways involved in the conversion of radiation energy to chemical energy (Moreland and Hilton, 1976).

Herbicides may be classified in several ways, according to the chemical compound, physiological characteristics or its selectivity (Percival and Baker, 1991). Herbicide can be classified into two major groups : organic and inorganic. Examples of organic herbicides are phenol and its derivatives, carboxylic acid and its derivatives,

pyridazine and pyrimidine, triazine, phenylurea, organophosphorus and organoarsenic compounds. Examples of inorganic herbicides are borate, sodium azide, sodium chlorate, copper sulfate and calcium cyanamide.

Herbicide action in general

Generally, the purpose of herbicides is to kill plant by inhibiting photosynthetic electron transport (Palmeira et al., 1995; Cobb, 1992). Two biochemical mechanisms have been demonstrated to be of primary importance in herbicidal action; they are firstly, inhibition of PSII electron transport and secondly, diversion of electron transfer through PSI (Fuerst and Norman, 1991). There are other mechanism of herbicides actions in plants. They are summarized in Table 1.

There are also herbicides that enhance the development of auxin, e.g., pheooxyalkanoic acid, benzoic acid, pyridine, and quinoline carboxilic acid (Cobb, 1992). Excessive development of auxin may cause the potassium ion accumulation in guard cells. Resulting in the opening of the stomata and an increase in the photosynthesis rate. Subsequently, cells die of excessive food production. Herbicide also

affect the increase of cell division and differentiation of cambium tissues in higher plants.

Table 1

Some examples of herbicide and their specific inhibition.

Inhibition	Herbicide	Reference
Photosynthesis		
PS1	Bipyridinium, Paraquat, Diquat	Dodge,1988
PS2	Urea, Triazines, Triazinones, Uracil	Cobb, 1992
Pigment biosynthesis	Pyridazinone, Metflurazone.	Britton et al. 1989.
Lipid synthesis	Cyclohexanadiones, Thiocarbamate	Cobb, 1992
Amino acid chain branching biosynthesis	Sulphonilurea, Imidazolinone, Sulphonanilides.	Dodge, 1988
Glutamine synthesis	Oxitin, Phosphinotrisine,	Cobb, 1992

Most of the chemical compounds that inhibit photosynthesis electron transport do so by binding to a set of overlapping sites on the photosystem II (PSII) reaction centre (Tietjen et al., 1991; Sandmann and Boger, 1986; Lavergne, 1982; Pfister et al., 1974). The phytotoxic symptoms arise from the interaction of the herbicide molecule with PSII. These chemical compounds include urea derivatives and triazines.

Urea derivatives and triazines were introduced between 1950 and 1960. Diuron was the first herbicide known to specifically inhibit photosynthetic electron flow (Wessels and Van der Veen, 1956). The site of inhibition at the acceptor site of PSII was proposed, based on the effect on chlorophyll fluorescence yield by Duysens and Sweers (1963). Since then, diuron, atrazine and their photoaffinity labelling analogues have been used extensively in photosynthesis research.

Triazine

Triazine herbicides were first introduced in 1955 by Geigy. Triazines are a commercially important class of herbicides. Triazine is an extreme volatile crystalline solid which melts at 86°C and boils at

114 °C at 1 atm pressure. It is easily soluble in ether and ethanol at -5°C.

Triazine is highly refracting rhombohedral crystals. The density is approximately 1.38 g/cm^3. Triazine can be purified without appreciable loss by repeated distillation over metallic sodium (Smolin and Rapoport, 1959).

Cyanuric chloric (2,4,6-trichloro-1,3,5,-triazine) is the base compound to form all 1,3,5-triazine herbicide. General chemical structure of triazine is shown in Figure 1.

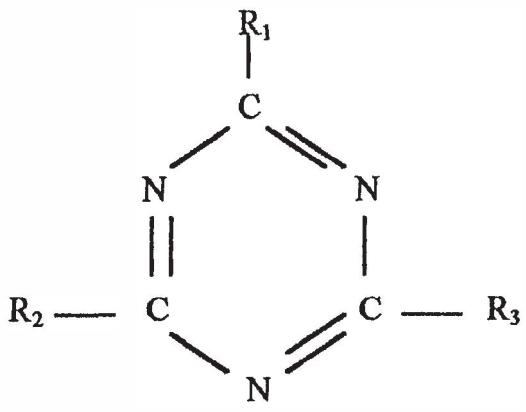

Figure 1: General structure of triazine.