



UNIVERSITI PUTRA MALAYSIA

**IMMOBILISATION AND CHARACTERISATION OF
CHLORELLA VULGARIS AS POTENTIAL BIO-INDICATOR
FOR SELECTED HERBICIDES**

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FSAS 2001 2

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By

SHAKINAZ DESA

**Thesis Submitted in Fulfilment of the Requirement for
the Degree of Doctor of Philosophy in the Faculty of
Science and the Environmental Studies
Universiti Putra Malaysia**

October 2001



DEDICATION

This work is dedicated to Associate Professor Dr. Nor Aripin Shamaan for his invaluable guidance throughout my years in UPM. I also dedicate this achievement to my beloved husband Zuhaidi Mukrim for his support and believing in me; to my son Muhammad Danish and my daughter Nabel Darwish, for cheering me up during the blues; to my mother Mariyah Ali, for blessing me and her prayers for my success; to my father Desa Hj. Demat for his blessing; to my sisters Desmariatul Safinaz and Shabrinaz, my brother Kamal Affendi for their inspirations and motivations.

I would also like to mention my fellow friends in the Department of Biochemistry and Microbiology, and Department of Biology as the most supportive group due to their helps and everlasting friendship. Lastly, I would like to dedicate this thesis to Universiti Putra Malaysia, which I have been studying in for almost 13 precious years, for the knowledge, experiences and memories.

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Dr. Nor Aripin Shamaan

Faculty: Science and Environmental Studies

Herbicides are one of the major contributing pollutants in water bodies. Several detection methods have been developed to monitor herbicide pollution including the use of bio-indicators. The competency of a bio-indicator in herbicide detection must comply with the sensitivity and efficiency of the method. In this study, *Chlorella vulgaris* as a bio-indicator was immobilised in alginate and compared with the free cell to determine its ability as a bio-indicator.

There were two immobilised conditions; immobilised cells of recommended cell concentration (2×10^4 cells/ml) and immobilised cells based upon suitability test. In the suitability test, four bead concentrations were tested; 0.1%w/w, 0.2%w/w, 0.4%w/w and 0.8%w/w. 0.1%w/w was selected as test bead based on stability and

water and 26 days in calcium chloride. The other bead concentrations were stable for less than 20 days. The 0.1%w/w bead had constant growth rate and exponential rate pattern of oxygen production for 7 days, compared with the other beads.

Free cells and two immobilised conditions were compared using two methods; oxygen production rate inhibition test and 96 hour's toxicity test. Four herbicides were used in this study; Atrazine, Simazine, Diuron and Paraquat. The first three are photosystem II inhibitor and Paraquat is a photosystem I inhibitor.

Immobilised microalgae was dark incubated in herbicide for 30 minutes before measuring the oxygen production rate. 30 minutes was chosen as incubation time due to significant inhibition of oxygen production rate by herbicide at this period. Light and temperature values during detection were previously examined and selected for suitability. The selected light intensity was $90\mu\text{mol}/\text{sec}/\text{m}^2$ and 28°C for sample chamber's temperature due to the production of oxygen at exponential rate.

Cells were incubated for 96 hours in herbicide with 12:12h light cycle for 96 hour's toxicity test. Cells were enumerated and compare to reference. For immobilised cells, cells were counted after dissolving the beads with trisodium citrate.

There were three significant findings in this study. First, the ability to immobilise *Chlorella vulgaris* as a 2mm bead, which can survive for more than three months. Second, immobilisation of the recommended cell number was the better choice as bio-indicator using oxygen production rate change compared to free cells or test bead. There was 50% inhibition using this condition at 0.12µM Atrazine, 5.8µM Simazine, 0.4µM Diuron and calculated value at 3.913 mM for Paraquat, while the other cell conditions needed higher concentration than 1000µM for 50% inhibition or could not exhibit 50% inhibition. Third, for toxicity testing, free cells is recommended compared to the immobilised cells. Toxicity of free cells at 1000µM was higher in Simazine > Atrazine > Diuron > Paraquat, while at 0.01µM: Diuron > Paraquat > Atrazine > Simazine. For the immobilised conditions, no 50% inhibition of cell number was observed, suggesting the cells were protected by alginate. In conclusion, immobilised cells are potential useful bio-indicator for herbicide or other pollutant that interfere with photosynthesis in water body. However, further research should be done to improve and simplify the method.

Abstrak tesis diserahkan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**IMMOBILISASI DAN PENCIRIAN *CHLORELLA VULGARIS* SEBAGAI
BIO-PENUNJUK BERPOTENSI BAGI RACUN RUMPAI TERPILIH**

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Racun rumpai adalah satu daripada bahan pencemaran di dalam air. Terdapat banyak kaedah pengesanan yang dibangunkan bagi membantu pemantauan di lapangan. Kebolehan bio-pengesanan di dalam mengesan racun rumpai mestilah selari dengan sensitiviti dan keberkesanan sesuatu kaedah. Di dalam kajian ini, *Chlorella vulgaris* sebagai bio-penunjuk dipegunkan di dalam alginate dan dibandingkan dengan keadaan bebasnya untuk mengetahui kebolehannya bertindak sebagai bio-penunjuk.

Terdapat dua keadaan pegun; memegun sel pada kepekatan yang disarankan (2×10^4 sel/ml) dan sel pegun kajian yang dipilih dari ujian kesesuaian. Di dalam ujian kesesuaian, empat kepekatan sel

dipilih sebagai sel pegun kajian berdasarkan kestabilan dan pertumbuhan yang sesuai. Sel pegun tersebut stabil selama 23 hari apabila disimpan di dalam air suling dan 26 hari di dalam kalsium klorida. Kepekatan sel pegun yang lain stabil selama kurang dari 20 hari. Sel pegun 0.1%w/w mempunyai pertumbuhan yang malar dan kadar eksponen bagi penghasilan oksigen selama 7 hari berbanding sel pegun yang lain..

Keadaan bebas dan pegun dibandingkan melalui dua ujikaji; Ujikaji perencatan kadar penghasilan oksigen dan Ujikaji ketoksikan 96 jam. Empat racun rumpai digunakan didalam kajian ini; Atrazine, Simazine, Diuron dan Paraquat. Tiga racun yang pertama adalah perencat fotosistem II manakala Paraquat adalah perencat fotosistem I.

Sel pegun dieram di dalam racun rumpai selama 30 minit di dalam keadaan gelap sebelum pengukuran kadar penghasilan oksigen dibuat. 30 minit dipilih sebagai masa pengeraman kerana perencatan oleh racun rumpai adalah signifikan pada jangkawaktu ini. Nilai cahaya dan suhu semasa pengukuran telah diuji dan dipilih mengikut kesesuaian terlebih dahulu. Nilai cahaya yang dipilih adalah $90\mu\text{mol}/\text{sec}/\text{m}^2$ and 28°C bagi suhu kebuk sampel berdasarkan kadar penghasilan oksigen yang eksponen.

Sel dieram selama 96 jam di dalam racun rumpai dengan 12:12j kitaran cahaya untuk ujian ketoksikan 96 jam. Sel dikira dan

dibandingkan dengan kawalan. Bagi sel pegun, sel dikira selepas diurai menggunakan trisodium sitrat.

Terdapat tiga penemuan yang signifikan di dalam kajian ini. Pertama, kebolehan untuk memegun *Chlorella vulgaris* bersaiz 2 mm diameter dan mampu hidup lebih dari tiga bulan. Kedua, sel pegun merupakan pilihan yang terbaik berbanding keadaan lain sebagai bio-penunjuk kepada perubahan kadar penghasilan oksigen berbanding sel bebas atau sel pegun kajian. Terdapat 50% perencatan pada 0.12 μ M Atrazine, 5.8 μ M Simazine, dan 0.4 μ M Diuron dan nilai pengiraan 3.913mM bagi Paraquat., manakala dua keadaan yang lain memerlukan kepekatan yang lebih tinggi dari 1000 μ M bagi perencatan 50% atau tidak boleh merencat 50%. Ketiga, bagi ujian ketoksikan, sel bebas disarankan penggunaannya berbanding sel pegun. Ketoksikan terhadap sel bebas pada 1000 μ M adalah tinggi di dalam Simazine > Atarazine > Diuron > Paraquat, manakala pada 0.01 μ M: Diuron > Paraquat > Atrazine > Simazine. Bagi kedua dua sel pegun, tidak terdapat 50% perencatan, menunjukkan sel dilindungi oleh alginate. Kesimpulannya, sel pegun berpotensi sebagai bio-penunjuk yang berguna terhadap racun rumpai atau pencemar lain yang mengganggu fotosintesis di dalam air. Bagaimana pun, kajian lanjutan mestilah dijalankan untuk memperbaiki dan memudahkan kaedah tersebut.

ACKNOWLEDGEMENTS

This study was completed under the supervision of Associate Professor Dr. Nor Aripin Shamaan (naripin@fsas.upm.edu.my) from the Department of Biochemistry and Microbiology. He has provided a superb supervision by allowing me to invent new technique independently, applying new ideas and to make things possible for the research. He had also given invaluable advice on the need of my research. I gratefully thank him for his efforts. Dr. Misri Kusnan (misri@fsas.upm.edu.my) and Dr. Hishamuddin Omar (hishamom@usa.net) were also involved in the success of the study by guiding and consulting this study. They had contributed their brilliant ideas, comments and tremendous support.

I would also express my gratitude to Professor Dr. Nora F.Y.Tam (bhntam@cityu.edu.hk) from City University of Hong Kong for contributing her ideas and comments on immobilisation of microalgae. She has many years of experience in microalgae and wastewater management. My grateful thanks also goes to Encik Khalid of Department of Biochemistry and Microbiology for letting me to occupy his laboratory for three and a half years; and to Encik Hidir of Department of Biology for allowing me to inoculate and producing axenic culture of *Chlorella vulgaris* in his well maintained laboratory. Finally, I would like to thank Universiti Putra Malaysia for providing the knowledge, facilities, convenience situation, motivation and inspiration throughout my study here.



I certify that an Examination Committee met on 19th October 2001 to conduct the final examination of Shakinaz Desa on her Doctor of Philosophy thesis entitled "Immobilisation and Characterisation *Chlorella vulgaris* as Potential Bio-indicator for Selected Herbicides" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. Members of the Examination Committee are as follows:


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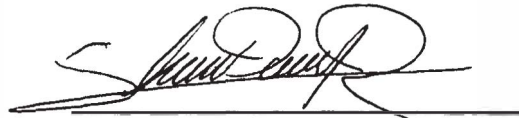
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions.



SHAKINAZ DESA

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TABLE OF CONTENTS

	PAGE
DEDICATION	2
ABSTRACT	3
ABSTRAK	6
ACKNOWLEDGEMENTS	9
APPROVAL SHEETS	10
DECLARATION FORM	12
LIST OF TABLES	16
LIST OF FIGURES	17
LIST OF ABBREVIATIONS	20
 CHAPTER	
I	
INTRODUCTION	21
Pesticide and Environment	21
Herbicide detection	24
Research's focus	26
Strategies and postulation	28
II	
LITERATURE REVIEW	30
Herbicide	30
Introduction	30
Herbicide action in general	31
Herbicide detection methods	32
The electron transport system and herbicide target sites	38
Photosystem I herbicide	42
Photosystem I	42
Paraquat	42
Mechanism of paraquat	44
Photosystem II herbicide	46
Triazine	46
Diuron	49
Photosystem II binding sites	51
The effect of herbicide application	54
Microalgae as a bio-indicator	60
<i>Chlorella vulgaris</i>	62
The ability of screening herbicides	64
The pollutant removal ability of <i>Chlorella</i>	65
Immobilisation	68

	Introduction	68
	Immobilisation of viable cells	70
	The cell entrapment	72
	The advantages and limitations of using immobilised cells	74
	The potential of immobilised <i>Chlorella sp.</i>	76
III	MATERIALS AND METHODS	79
	Objective and rationale	79
	General experimental design	80
	Preparation of <i>Chlorella vulgaris</i> 's stock culture ..	82
	Preparation of media	82
	Growing and maintaining <i>C. vulgaris</i>	83
	Cells density determination	85
	Preparation of Test Bio-indicator	86
	Free cells and standard bead preparation	87
	Test bead preparation	87
	Immobilisation of <i>Chlorella vulgaris</i> using alginate ...	88
	Preparation of alginate	88
	Preparation of <i>Chlorella vulgaris</i>	89
	Bead preparation	89
	Bead concentration determination	90
	Bead stability	92
	Preparation of herbicide	93
	The basic principle of oxygen detector	95
	Standard value determination	96
	Oxygen production rate determination	97
	Parameter selection for oxygen production rate detection	98
	Light intensity selection	98
	Temperature selection	99
	Herbicide reacting time experiment	99
	Comparative experiments	100
	Herbicide inhibition	100
	96 hours toxicity test	100
	Statistical and data analysis	102
IV	RESULTS AND DISCUSSION	103
	The immobilisation of <i>Chlorella vulgaris</i>	103
	Factors that contribute to the beads development	103
	Technique of extruding suspension into cross-linking media	107
	Preliminary study: height of extrusion tip	107
	Precaution during immobilisation	108

Cell peripheral growth in bead	109
Selection of bead concentration for test bead bio-indicator	110
.....	
Test bead selection: stability of test bead in storing media	110
Test bead selection: oxygen production rate Monitoring	115
Test bead selection: Initial cell density load determination	116
The oxygen production rate and cell growth observation in different media	117
Oxygen production rate and cell growth in distilled water	118
The possibility of microenvironment change to alter microalgae's response	121
Parameter optimisation for oxygen detection	129
Light intensity	129
Light intensity towards free cells	130
Light intensity towards standard beads	134
Light intensity towards test beads	135
Summary of light intensity selection	136
Temperature selection	144
Summary of temperature selection	149
Incubation time of test bio-indicator in herbicides	153
Summary of incubation time selection	167
Herbicide inhibition	168
Atrazine inhibition	169
Simazine inhibition	174
Diuron inhibition	178
Paraquat inhibition	182
The 96 hours toxicity test	187
Toxicity on free cells	187
Toxicity on standard beads	189
Toxicity on test bead	192
Summary of toxicity test	194
	196
V CONCLUSION	
Immobilisation	196
Oxygen yield detection	197
Herbicide action	198
Potential and suggestions	198

REFERENCES	201
APPENDICES	
A Bold Basal Media	221
B1 Action by Atrazine	222
B2 Action by Simazine	223
B3 Action by Diuron	224
B4 Action by Paraquat	225
BIOGRAPHICAL SKETCH	226

LIST OF TABLES

Table		Page
1	The comparison between conventional methods and biological based analyses	26
2	Some examples of herbicide and their specific inhibition	32
3	The basic physiological facts of <i>Chlorella vulgaris</i>	63
4	The study of metallic species using <i>Chlorella vulgaris</i>	67
5	Examples of cells immobilised by entrapment method	74
6	Basic information on the test herbicide	94
7	Statistical analyses of cell number and oxygen production rates in three different bead concentrations	118
8	Statistical analyses of cell number and oxygen production rates of beads in Calcium chloride media	126
9	The different of oxygen production rate between three cell condition at 90 $\mu\text{mol}/\text{m}^2/\text{sec}$ photon flux density	143
10	The comparison of oxygen production rate at 32°C	150
11	The ability of three cell conditions to exhibit 50% inhibition of oxygen production rate due to herbicide within the range of 0.01 μM to 1000 μM	186
12	A 50% inhibition of cell number by test herbicide on three cell conditions	195
13	The composition of Bold Basal Media	221

LIST OF FIGURES

Figure		Page
1	The diagram represents electron transport system inside the thylakoid membrane, which involves two photosystems	39
2	The structure of paraquat ion	43
3	The sequence of electron flow from FeS to either paraquat or ferredoxin	45
4	General structure of triazine	48
5	Structure of diuron or DCMU	50
6	How a contaminant can enter water body	54
7	Schematic representation of cell immobilisation	72
8	Summary of general experimental design	81
9	The cup shaped chloroplast of <i>Chlorella vulgaris</i> can be seen clearly from under 100x magnification	84
10	Cool white fluorescence was used as light source and was arranged in such way to ensure equal light exposure	84
11	Summary of test bio-indicators preparation	86
12	The procedure of immobilising microalgae in alginate	91
13	The extrusion of cell-alginate suspension into calcium chloride to form beads	92
14	Example of a standard value data recorded on a chart graph ..	96
15	Example of an oxygen detector response from a sample on a chart graph	97
16	The replacement of free cell (a) and immobilised cells (b) in the incubation chamber	101

17	The view of cells entrapped in alginate matrix (40x)	104
18	<i>Chlorella vulgaris</i> beads formed	104
19	Different bead formation due to stirring speed in cross linking media	106
20	The peripheral zone of cell growth in beads of more than 2 weeks old	109
21	a) Bead rupture	112
	b) Stability of alginate beads	113
22	Oxygen production rate and cell growth in distilled water	119
23	Blank alginate beads are slightly opaque	122
24	Weekly monitoring of oxygen production of bead in distilled water revealed a reduction for 0.4%w/w and 0.1%w/w	124
25	Oxygen production rate stored in Calcium chloride	127
26	Oxygen production rate due to the exposure of different light intensity	131
27	Oxygen production rate due to the exposure of different temperature	145
28	Inhibition of oxygen production rate due to 1000 μ M herbicide incubation time	155
29	Inhibition of oxygen production rate due to 1 μ M herbicide incubation time	162
30	Inhibition of oxygen production rate due to 0.01 μ M herbicide incubation time	166
31	Percentage of oxygen production inhibition by Atrazine	170
32	The comparison of determined percentage using two different cell conditions to the referral dosage of permitted level in drinking water	174
33	Percentage of oxygen production inhibition by Simazine	176

34	Percentage of oxygen production inhibition by Diuron	179
35	Percentage of oxygen production inhibition by Paraquat	183
36	96 hours toxicity test: free cells of <i>Chlorella vulgaris</i> was incubated in different herbicides and cell number were counted after 96 hours with the comparison using its control	188
37	96 hours toxicity test: standard beads of <i>Chlorella vulgaris</i> was incubated in different herbicides and cell number were counted after 96 hours with the comparison using its control	190
38	96 hours toxicity test: test beads of <i>Chlorella vulgaris</i> was incubated in different herbicides and cell number were counted after 96 hours with the comparison using its control	193
39	Action by Atrazine	222
40	Action by Simazine	223
41	Action by Diuron	224
42	Action by Paraquat	225

LIST OF ABBREVIATIONS

BBM	Bold basal media
EPA	Environmental Protection Agency
PSI	Photosystem I
PSII	Photosystem II

CHAPTER 1

INTRODUCTION

Pesticides And Environment

Rapid increase in world population has led to intensive farming as the better option for food production. Inevitably, a successful intensive farming requires extensive use of agrochemicals. Numerous studies have been carried out worldwide which indicated that the extensive use of pesticides has directly or indirectly caused adverse effects to the environment.

Most of the agrochemicals are inherently toxic to living organisms and inevitably affect human health. The indiscriminate use of pesticides not only affects human health but also creates serious environmental implication. However, the effects of herbicide contamination on aquatic systems depend on the characteristics of the herbicide, its concentration and the nature and biology of the aquatic systems.

The pesticide industry in Malaysia is heavily dependent on imported active ingredients and foreign product technology (Yeoh et al., 1991). This is because pesticides play an important role in crop

protection for the foreseeable future, as there are no practical alternatives at the moment. However, there are tremendous changes in some of the latest pesticides introduced; for instance the significant reduction in dosage rates and their reduced persistence in the environment.

Pesticides enter water body easily via soil leaching, spray drift or through ground water. Besides containing aquatic food chain, water is important and its scarce resource has been used for irrigation, aquaculture and human consumption. Therefore it is vital to keep water sources safe. Thus, pollution monitoring and treatment become an important issue. It brings on researchers throughout the world to develop methods of herbicide detection in water. Their goal is to produce a fast, cheap, easy-handling and reliable pesticides-detecting tool.

Herbicide Detection

Current herbicide detection methods are not perfectly suitable for large scale monitoring or field monitoring programme. These methods include gas chromatography (GC) and high performance liquid chromatography (HPLC) that is affordable by most laboratories. The techniques required large sample volumes, extensive extraction and clean up procedures for analyses. These techniques also require

solvents that will finally end up polluting the environment. The chemical analyses carried out in laboratory are laborious and expensive.

Nowadays there are high-speed techniques that may detect heavy metals and pesticides. For example, induced couple plasma mass spectrometry (ICPMS) and gas chromatography mass spectrometry (GCMS) are among the most reliable equipment in Malaysia. However, the drawback is the cost of the equipment. Detection can only be carried out by research institutes, government based monitoring team or private companies that owns the facilities. Therefore it is useful to develop a reliable detecting method that is affordable by all users.

The accuracy, reproducibility and sensitivity of conventional methods and biological based methods are almost of the same quality (Korpan and El'skaya, 1995; Pandard and Rawson, 1993 and Gaisford et al., 1991). Table I summarises the comparison of conventional methods and biological based analyses on several aspects. The biological based analyses mainly involved the usage of microalgae, plant organelles and bacteria.

The basis of any detection tool is accuracy, precision, repeatability, reproducibility, sensitivity and reliability. Therefore, development of any detection method for herbicides should consider the