



**UNIVERSITI PUTRA MALAYSIA**

**RECOVERY OF ANTHRAQUINONES FROM *MORINDA ELLIPTICA*  
CELL CULTURE VIA *IN SITU* ADSORPTION USING POLYMERIC  
ADSORBENTS**

**CHIANG LIM**

**FSTM 2007 6**

**RECOVERY OF ANTHRAQUINONES FROM *MORINDA ELLIPTICA* CELL  
CULTURE VIA *IN SITU* ADSORPTION USING POLYMERIC ADSORBENTS**

**By**

**CHIANG LIM**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Master of Science**

**January 2007**



*This dissertation is especially dedicated to*

*my loving family and*

*my dearest friends who believe in me.....*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**RECOVERY OF ANTHRAQUINONES FROM *MORINDA ELLIPTICA* CELL CULTURE VIA *IN SITU* ADSORPTION USING POLYMERIC ADSORBENTS**

By

**CHIANG LIM**

**January 2007**

**Chairman: Associate Professor Badlishah Sham Baharin**

**Faculty: Food Science and Technology**

*Morinda elliptica* (Rubiaceae) cell suspension culture was used as a model system to understand the effects of *in situ* adsorption by polymeric adsorbents. The adsorption capacities of the adsorbents were determined and their equilibrium adsorption were fitted to Langmuir, Freundlich and Redlich-Petersen isotherms using linear and non-linear methods of analyses. The kinetic profiles of cell growth and anthraquinone (AQ) production were determined for cultures grown in intermediary (G) and production (P) medium strategies. Selection of the most suitable solvent was also carried out for effective recovery of AQ from the adsorbents. Co-cultivation of both untreated and pretreated adsorbents with G and P medium cultures were carried out to select a more biocompatible adsorbent that could enhance AQ production without affecting cell growth. The selected adsorbents were then further investigated for effective *in situ* adsorption factors in P medium strategies. High performance liquid chromatography (HPLC) was used for qualitative analyses of AQ constituents for extracts obtained from cells, culture medium and adsorbents.



XAD-16 showed the highest capacity at 0.0424mg alizarin/mg adsorbents whereas XAD-4 and XAD-7 showed a capacity of 0.0113 and 0.0109mg alizarin/mg adsorbents at initial alizarin solution concentration of 200mg/L, respectively. Freundlich isotherm fitted well to both XAD-4 and XAD-7 whereas Langmuir isotherm gave the best correlation to XAD-16 over the concentration ranges studied.

Ethanol was chosen as the eluting solvent with highest AQ recovery at 11.13mg/g, 5.20mg/g and 4.92mg/g eluted from XAD-4, XAD-7 and XAD-16, respectively. *M. elliptica* cell cultures achieved the highest biomass concentration at 36.79g/L on day 18 with 13.49mg/g DW intracellular AQ obtained in G medium strategy. In P medium strategy, the biomass concentration peaked on day 21 at 48.37g/L with intracellular AQ production recovered at 117.81mg/g DW. As 0.15g of both pretreated and untreated resins were added into cell cultures on day 15 and harvested on day 21, sodium acetate-pretreated XAD-4 stimulated AQ production to the highest extent in both G and P medium cultures. In G medium cultures, 25.67mg/g intracellular AQ was obtained, which was 1.4-fold to control. 1.04mg/L AQ recovered from the culture medium was 1.6-fold to control whereas 0.97mg/g AQ was obtained from the resins. Cell growth was comparable to control. In P medium cultures, cell growth was retarded where 15.43g/L biomass concentration were obtained, which was 23% lower than control. However, as high as 76.21mg/g intracellular AQ was obtained, which marked 1.4-fold increase to control. While 12.21mg/L extracellular AQ recovered was 6.6-fold higher than control, 1.08mg/g AQ was recovered from the resins.



When treated with 0.15g sodium acetate-pretreated XAD-4 on day 18, cell growth was comparable to control after 6 days of co-cultivation. 123.83mg/g DW intracellular AQ was obtained, which was 1.7-fold to control. 14.34mg/L extracellular AQ was recovered, which was 11-fold to control, whereas 2.7mg/g AQ was desorbed from the resins. When the factors were further studied, as high as 68.99mg/g DW intracellular AQ was obtained when cultures were treated with 0.25g XAD-4 on day 18 and harvested on day 24. This was 1.2-fold higher than control. 6.32mg/L extracellular AQ was recovered, which was comparable to control, while 0.52mg/g AQ was desorbed from the resins. However, cell growth was reduced 9.5% to 34.77g/L compared to control. A few types of AQ constituents were detected from the cells, culture medium and XAD-4 resins through qualitative HPLC analyses. Four different types of AQ compound were identified. While only rubiadin-1-methyl ether was detected in the cells, both damnacanthal and nordamnacanthal were detected from the culture medium whereas lucidin- $\omega$ -methyl ether was detected from XAD-4 resins. Numerous unidentified peaks were also detected frequently from the AQ extracts.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGUMPULAN ANTHRAKUINON DARI AMPAIAN SEL *MORINDA*  
*ELLIPTICA* SECARA PENJERAPAN “*IN SITU*” DENGAN MENGGUNAKAN  
PENJERAP**

Oleh

**CHIANG LIM**

**Januari 2007**

**Pengerusi: Profesor Madya Badlishah Sham Baharin**

**Fakulti: Sains dan Teknologi Makanan**

Ampaian sel *Morinda elliptica* (Rubiaceae) telah digunakan sebagai sistem model untuk memahami kesan penjerapan secara “*in situ*” oleh penjerap. Kapasiti penjerapan penjerap ditentukan dan penjerapan penjerap pada keseimbangan telah ditentukan dengan isoterma Langmuir, Freundlich dan Redlich-Petersen melalui kaedah analisis linear dan bukan-linear. Profil kinetik pertumbuhan sel dan penghasilan anthrakuinon (AQ) ditentukan daripada sel kultur yang tumbuh di dalam strategi media perantaraan (G) dan penghasilan (P). Pemilihan pelarut yang paling sesuai untuk pengumpulan anthrakuinon yang dijerap pada permukaan penjerap juga dijalankan. Penjerap yang dirawat dan yang tidak dirawat dikultivasi bersama dengan sel kultur untuk memilih penjerap yang dapat meningkatkan penghasilan anthrakuinon tanpa merencatkan pertumbuhan sel di dalam strategi media G dan P. Penjerap yang terpilih kemudian digunakan untuk mengkaji factor penjerapan secara “*in situ*” yang berkesan di dalam strategi media P. Teknik kromatografi cecair bertekanan tinggi (HPLC) digunakan untuk analisis komponen-komponen anthrakuinon yang diperolehi dari sel, media kultur dan penjerap secara kualitatif.



Dengan permulaan kepekatan larutan alizarin pada 200mg/L, XAD-16 menunjukkan kapasiti penjerapan yang tertinggi pada 0.0424mg alizarin/mg penjerap manakala XAD-4 dan XAD-7 menunjukkan kapasiti penjerapan pada 0.0113 dan 0.0109mg alizarin/mg penjerap. Isoterma Freundlich dapat disesuaikan kepada XAD-4 dan XAD-7 manakala isoterma Langmuir memberikan korelasi yang paling sesuai kepada XAD-16 dalam lingkungan kepekatan yang dikaji.

Etanol dipilih sebagai pelarut AQ daripada penjerap memandangkan 11.13mg/g, 5.20mg/g dan 4.92mg/g AQ diperolehi daripada XAD-4, XAD-7 dan XAD-16. Pada hari ke-18, kultur sel *M. elliptica* mencapai pertumbuhan sel yang tertinggi pada 36.79g/L dan 13.49mg/g (berat kering) DW kandungan AQ intrasel di dalam strategi media G. Di dalam strategi media P, pertumbuhan sel mencapai tahap tertinggi pada 48.37g/L dan 117.81mg/g DW kandungan AQ intrasel diperolehi pada hari ke-21. Apabila 0.15g penjerap yang dirawat dan yang tidak dirawat dimasukkan ke dalam kultur sel pada hari ke-15 dan dianalisis pada hari ke-21, XAD-4 yang dirawat dengan larutan natrium asetat meningkatkan penghasilan AQ yang tertinggi di dalam strategi media G dan P. Di dalam strategi media G, 25.67mg/g AQ intrasel diperolehi, iaitu 1.4 kali ganda lebih tinggi dari kawalan. 1.04mg/L AQ diperolehi dari media kultur, iaitu 1.6 kali ganda lebih tinggi dari kawalan, manakala 0.97mg/g AQ diperolehi dari penjerap. Pertumbuhan sel adalah setara dengan kawalan. Di dalam strategi media P, pertumbuhan sel terencat di mana 15.43g/L biomas sel diperolehi, iaitu 23% peratus lebih rendah dari kawalan. Walau bagaimanapun, 76.21mg/g AQ intrasel masih diperolehi, iaitu 1.4 kali ganda lebih tinggi dari control. 12.21mg/L AQ luar sel



diperolehi, iaitu 6.6 kali ganda lebih tinggi dari kawalan manakala 1.08mg/g AQ diperolehi dari resin.

Apabila dirawat dengan 0.15g XAD-4 yang dirawat dengan larutan natrium asetat pada hari ke-18, pertumbuhan sel adalah setara dengan kawalan selepas kultivasi bersama selama 6 hari. 123.83mg/g DW AQ intrasel diperolehi, iaitu 1.7 kali ganda lebih tinggi dari kawalan. 14.34mg/L AQ luar sel dikumpul, iaitu 11 kali ganda lebih tinggi dari kawalan, manakala 2.7mg/g AQ diperolehi dari penjerap. Apabila faktor-faktor dikaji dengan lebih mendalam, 68.99mg/g DW AQ intrasel diperolehi di dalam kultur sel yang dirawat dengan 0.25g XAD-4 pada hari ke-18 dan dianalisis pada hari ke-24. Ini adalah 1.2 kali ganda lebih tinggi dari kawalan. 6.32mg/L AQ luar sel dikumpul, iaitu setara dengan kawalan, manakala 0.52mg/g AQ diperolehi dari penjerap. Walau bagaimanapun, pertumbuhan sel dikurangkan sebanyak 9.5% kepada 34.77g/L jika dibandingkan dengan kawalan. Beberapa jenis komponen AQ dikesan dari sel, media kultur dan XAD-4 melalui analisis HPLC secara kualitatif. Empat jenis komponen AQ dapat dikesan. Rubiadin-1-metil eter hanya dikesan dalam sel, damnakantal dan nordamnakantal dikesan di dalam media kultur, manakala lusidin- $\omega$ -metil eter dikesan dari resin XAD-4. Beberapa komponen yang tidak dikenali juga kerap dikesan dari ekstrak AQ.

## ACKNOWLEDGEMENTS

First and foremost, I would like to convey my utmost gratitude to Assoc. Prof. Badlishah Sham Baharin and Dr. Mohd. Azmuddin Abdullah for their endless dedication, insight and enthusiasms, unfailing patience, encouragement, invaluable guidance, constructive comments and ideas, and critical reading of this thesis which contributed to its successful completion. My sincere appreciation to Assoc. Prof. Dr. Lai Oi Ming and Assoc. Prof. Dr. Thomas Choong Shean Yaw for their endless support, suggestions and advices throughout my project and thesis writing. To Mr. Ong, En. Rosli and Kak Ima – thanks for all the assistance. My special appreciation extended to the Malaysian Government for the PASCA scholarship that has enabled me to complete my study without any financial worries. To my labmates – Kak Rozita, Tzer Miin, Lin, Su, Ziha, Susan and Bobby. Thanks for making this project an enjoyable one. Not forgetting the members of Plant Tissue Culture Lab at MKT, UPM – Kak Nor, Kak Ummi, Mei Kying, Thuc and Mai Anh – we had been through our toughest challenge during our studies, yet we had come this far, hand in hand. I was truly grateful to have you all by my side. To KC and Cam, thanks for the help that I needed in my HPLC analyses. And of course to Gan, Wai Cheng, Tuck Keong, Hwee Nee, Helen, Grace and others whom I may have not listed here. Thanks for giving me a place to lean on and pouring your continuous support, encouragement, strength and patience in every twist and turn that I had endured all these whiles. Words could not describe how much it meant to me. Those moments will always be a walk to remember in my life. You all will always have a place in my heart. Last but not least, my heartfelt gratitude to my family for lifting me up whenever I needed those extra boosts, needless to say, your love, support and encouragement.



I certify that an Examination Committee has met on **8<sup>th</sup> January 2007** to conduct the final examination of Chiang Lim on her Master of Science thesis entitled “Recovery of Anthraquinones via *in situ* Adsorption by Polymeric Adsorbents in *Morinda elliptica* Cell Suspension Cultures” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Suraini Abd. Aziz, PhD**

Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Arbakariya Ariff, PhD**

Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Rosfarizan Mohamad, PhD**

Lecturer  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Abdul Wahab Mohamad, PhD**

Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(External Examiner)

---

**HASANAH MOHD. GHAZALI, PhD**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**Badlishah Sham Baharin**

Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Chairman)

**Lai Oi Ming, PhD**

Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

**Thomas Choong Shean Yaw, PhD**

Associate Professor  
Faculty of Engineering  
Universiti Putra Malaysia  
(Member)

**Mohd. Azmuddin Abdullah, PhD**

Senior Lecturer  
Department of Chemical Engineering  
Universiti Teknologi Petronas  
(Member)

---

**AINI IDERIS, PhD**

Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 10 MAY 2007



## **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 other degree at UPM or other institutions.

---

**CHIANG LIM**

Date: before 27 APRIL 2007



## TABLE OF CONTENTS

	Page
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	ix
<b>APPROVAL</b>	x
<b>DECLARATION</b>	xii
<b>LIST OF TABLES</b>	xvi
<b>LIST OF FIGURES</b>	xviii
<b>LIST OF PLATES</b>	xxi
<b>LIST OF ABBREVIATIONS</b>	xxii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	1
<b>2 LITERATURE REVIEW</b>	8
2.1 Herbaceous Plants in Malaysia	8
2.2 <i>Morinda elliptica</i> and Biological Activities of Anthraquinones	10
2.3 Plant Primary and Secondary Metabolites	15
2.4 Plant Cell Culture as a Source of Secondary Metabolite Production	16
2.5 Challenges Facing Plant Cell Culture Technology	28
2.6 Transport Mechanisms in Cultivated Plant Cells	31
2.6.1 Active Transport	35
2.6.2 Passive (diffusion) Transport	39
2.6.3 Transport Mechanism in Two-phase Systems	40
2.7 Methods for Product Recovery from Plant Cell Cultures	42
2.7.1 Product Recovery <i>via</i> Liquid Phase Extraction	42
2.7.2 Product Recovery <i>via</i> in situ Adsorption	49
2.8 Microporous Adsorbents	53
2.9 Polymeric Adsorbents	55
2.10 Adsorption Mechanisms and Isotherms	58
2.10.1 The Langmuir Isotherm	60
2.10.2 The Freundlich Isotherm	61
2.10.3 The Redlich-Petersen Isotherm	62
2.11 Integrated Bioprocess Engineering	63
<b>3 ADSORPTION CAPACITY AND ADSORPTION ISOTHERMS OF ALIZARIN ONTO AMBERLITE POLYMERIC ADSORBENTS</b>	68
3.1 Introduction	68
3.2 Materials and Methods	69
3.2.1 Adsorbents	69



3.2.2	Adsorption Equilibrium and Efficiency	70
3.2.3	Adsorption Isotherm Models	70
3.2.4	Statistical Analysis	71
3.3	Results and Discussion	72
3.3.1	Adsorption Capacity	72
3.3.2	Equilibrium Adsorption Isotherm Using Linear and Non-linear method	75
3.4	Conclusion	82
<b>4</b>	<b>CO-CULTIVATION OF AMBERLITE POLYMERIC ADSORBENTS WITH <i>MORINDA ELLIPTICA</i> CELL SUSPENSION CULTURE</b>	<b>83</b>
4.1	Introduction	83
4.2	Materials and Methods	85
4.2.1	Cell Suspension Culture	85
4.2.2	Analytical Procedures	86
4.2.2	Profiles of <i>Morinda elliptica</i> Cell Suspension Cultures	87
4.2.4	Co-cultivation of <i>Morinda elliptica</i> Cell Cultures and Adsorbents	88
4.2.4.1	Desorption Analysis	88
4.2.4.2	Effects of Adsorbent Pretreatment	88
4.2.5	Determination of Rate of Product Formation	89
4.2.6	Statistical Analysis	89
4.3	Results and Discussion	91
4.3.1	Time Course of Cell Growth and Total Anthraquinone (AQ) Production of <i>Morinda elliptica</i> Cell Cultures in Intermediary (G) and Production (P) Medium	90
4.3.2	Co-cultivation of <i>Morinda elliptica</i> Cell Suspension Cultures with Different Polymeric Adsorbents	97
4.3.2.1	Desorption Analysis	97
4.3.2.2	Effects of Adsorbent Pretreatment	102
4.4	Conclusion	113
<b>5</b>	<b>EFFECTS OF DAY OF TREATMENT, AMOUNT OF ADSORBENTS AND CONTACT PERIODS FOR ANTHRAQUINONE ADSORPTION IN <i>MORINDA ELLIPTICA</i> CELL CULTURES</b>	<b>114</b>
5.1	Introduction	114
5.2	Materials and Methods	115
5.2.1	Cell Suspension Cultures	115
5.2.2	Analytical Procedures	116
5.2.3	HPLC System	116
5.2.4	Preparation of Sample Extracts for HPLC Analyses	117



5.2.5	Statistical Analysis	117
5.3	Results and Discussion	118
5.3.1	Effect of Day of Treatment	118
5.3.2	Effects of The Amount of XAD-4 Adsorbents and Contact Periods	125
5.3.3	Qualitative HPLC Analyses of Anthraquinone Constituents	132
5.4	Conclusion	144
<b>6</b>	<b>GENERAL DISCUSSION, CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK</b>	145
6.1	Adsorption Capacity and Adsorption Isotherms of Alizarin Adsorption onto Amberlite Polymeric Adsorbents	145
6.2	Co-cultivation of Polymeric Adsorbents with <i>Morinda elliptica</i> Cell Suspension Cultures	146
6.3	Suggestions for Future Work	148
	<b>REFERENCES</b>	150
	<b>APPENDICES</b>	166
	<b>BIODATA OF THE AUTHOR</b>	180





## LIST OF TABLES

Table		Page
2.1	Commonly known herbaceous plants in Malaysia	9
2.2	Plant-derived products of pharmaceutical importance	17
2.3	Groups of natural products that have been isolated from tissue and cell suspension cultures of higher plants	19
2.4	Food additives from plant cell cultures	20
2.5	Product yields from cell cultures and whole plant	22
2.6	High yields of secondary products from cell suspension, callus and immobilized cultures	23
2.7	Strategies to enhance production of secondary metabolites in plant cell cultures	26
2.8	Large-scale reactors for plant cell suspension cultures	27
2.9	Bioreactor types used for plant cell cultures	27
2.10	Log <i>P</i> values of the organic solvents	48
2.11	Desired characteristics of a second phase for effective product accumulation	52
2.12	Selection criteria for solid adsorbents for <i>in situ</i> product-removal applications	59
2.13	General features of physical adsorption and chemisorption	59
3.1	Typical properties of Amberlite polymeric adsorbents and alizarin adsorption efficiency	74
3.2	Isotherm constants for alizarin sorption onto XAD-4, XAD-7 and XAD-16 resins using linear method	78
3.3	Isotherm constants for alizarin sorption onto XAD-4, XAD-7 and XAD-16 resins using non-linear method	81
3.4	$Q_e$ and $C_e$ values for XAD-4, XAD-7 and XAD-16 resins	169



4.1	Medium formulation for maintenance (M), intermediary (G) and production (P) medium with increased strength (g/L)	168
4.2	Amount of AQ (mg/g) desorbed from varying adsorbents using different types of solvents	170
4.3	Effects of 0.15g untreated and pretreated adsorbents on G medium <i>M. elliptica</i> cell suspension cultures treated on day 15 and cultures harvested on day 21	172
4.4	Effects of 0.15g untreated and pretreated adsorbents on P medium <i>M. elliptica</i> cell suspension cultures treated on day 15 and cultures harvested on day 21	173
4.5	Effects of nylon sachets on G and P medium <i>M. elliptica</i> cell suspension cultures added on day 15 and cultures harvested on day 21	174
5.1	Effects of day of adsorbent treatment on <i>M. elliptica</i> cell suspension cultures harvested after 6 days of treatment	175
5.2	Effects of contact period and varying amount of pretreated XAD-4 on <i>M. elliptica</i> cell suspension cultures	176
5.3	Effects of XAD-4 resins on intracellular AQ constituents and unknown compounds of <i>M. elliptica</i> cell suspension cultures treated on day 18	177
5.4	Effects of XAD-4 resins on extracellular AQ constituents and unknown compounds of <i>M. elliptica</i> cell suspension cultures treated on day 18	178
5.5	AQ constituents and unknown compounds adsorbed onto XAD-4 resins in <i>M. elliptica</i> cell suspension cultures treated on day 18	179



## LIST OF FIGURES

Figure		Page
2.1	Basic structure of anthraquinones isolated from the roots and cell suspension cultures of <i>Morinda elliptica</i>	14
2.2	Storage compartments for hydrophilic and lipophilic compounds	33
2.3	Transportation of secondary metabolites in (a) storage (b) secretion (c) uptake	34
2.4	(a) Active transport and (b) passive transport mechanisms in plant cells, <i>in vitro</i>	37
2.5	Primary and secondary transports across the plasma membrane	38
2.6	The presence of second artificial accumulation phase and the interaction of many different factors that control the transport of secondary metabolites as well as other compounds	41
2.7	Chemical structure of AMBERLITE XAD-4 and XAD-16 (Rohm & Haas Co.) polymeric adsorbents	57
2.8	Chemical structure of AMBERLITE XAD-7 (Rohm & Haas Co.) polymeric adsorbents	57
3.1	Equilibrium adsorption isotherms for alizarin on XAD polymeric adsorbents at $24\pm 2^{\circ}\text{C}$	74
3.2	(A) Langmuir, (B) Freundlich, and (C) Redlich-Petersen isotherm linear plots for the sorption of alizarin onto XAD-4, XAD-7 and XAD-16 resins	77
3.3	Equilibrium curves for sorption of alizarin onto XAD-4, XAD-7 and XAD-16 resins using linear method	78
3.4	Equilibrium curves for sorption of alizarin onto XAD-4, XAD-7 and XAD-16 resins using non-linear method	81
4.1a	The profile of cell growth, pH, intracellular AQ content, extracellular AQ and total AQ in G medium strategy of <i>M. elliptica</i> cell suspension cultures	95



4.1b	The profile of cell growth, pH, intracellular AQ content, extracellular AQ and total AQ in P medium strategy of <i>M. elliptica</i> cell suspension cultures	96
4.2	Amount of AQ recovered from the polymeric adsorbents using organic solvents of different polarities	101
4.3	The effects of pretreated polymeric adsorbents (Control, XAD-4, XAD-7, XAD-16) on pH, cell growth, and intracellular AQ content in <i>M. elliptica</i> cell suspension cultures in both G and P medium	107
4.4	The effects of pretreated polymeric adsorbents (Control, XAD-4, XAD-7, XAD-16) on extracellular AQ, amount of AQ adsorbed and total AQ in <i>M. elliptica</i> cell suspension cultures in both G and P medium	112
4.5	Standard curve for intracellular AQ dissolved in dichloromethane (Abs 420 nm) and extracellular AQ dissolved in 80% methanol (Abs 435 nm)	167
4.6	Plot $dP/dt$ vs $dX/dt$ and $X$	171
5.1	The effects of pre-treated XAD-4 and XAD-16 on pH, cell growth, intracellular AQ content, extracellular AQ titre, AQ adsorbed, and total AQ in <i>M. elliptica</i> cell suspension cultures, treated on different stages of cell growth cycle in P medium compared to control	124
5.2	The effects of sodium acetate-pre-treated XAD-4 and day of culture harvesting on pH, cell growth, intracellular AQ, extracellular AQ, amount of AQ adsorbed, and total AQ in <i>M. elliptica</i> cell suspension cultures in P medium	131
5.3	HPLC chromatographs of external standards, reference and cell culture extracts of <i>M. elliptica</i> treated with varying amount of XAD-4 resins on day 18, and cultures harvested after 4 days	134
5.4	Effects of varying amount of XAD-4 and day of culture harvesting on the AQ and unknown compounds of intracellular AQ extracts of <i>M. elliptica</i> cell cultures in P medium for treatment on day 18	135
5.5	HPLC chromatographs of external standards and AQ extracts recovered from culture medium of <i>M. elliptica</i> treated with varying amount of XAD-4 resins on day 18	138



5.6	Effects of varying amount of XAD-4 and day of culture harvesting on the AQ and unknown compounds of extracellular AQ extracts of <i>M. elliptica</i> cell cultures in P medium for treatment on day 18	139
5.7	HPLC chromatographs of external standards and AQ extracts recovered from XAD-4 co-cultivated in <i>M. elliptica</i> cell cultures treated on day 18	142
5.8	Effects of varying amount of XAD-4 and day of culture harvesting on more frequently appearing AQ and unknown compounds of AQ extracts desorbed from XAD-4 co-cultivated in <i>M. elliptica</i> cell cultures in P medium for treatment on day 18	143



## LIST OF PLATES

Plate		Page
1	<i>M. elliptica</i> tree at the Institute of Bioscience, Universiti Putra Malaysia	11
2	Leaves of <i>M. elliptica</i>	11
3	Harvested cells of <i>M. elliptica</i> after 7 days of cultivation in (a) Maintenance (M), (b) Intermediary (G), and (c) Production (P) medium	91
4	The colour of XAD-4, XAD-7 and XAD-16 resins (after drying) before co-culturing in <i>M. elliptica</i> suspension cultures	100
5	The colour of XAD-4, XAD-7 and XAD-16 resins after harvesting from <i>M. elliptica</i> suspension cultures	100
6	Control and treated <i>M. elliptica</i> cell suspension cultures in G and P medium after 7 days of cultivation	103



## LIST OF ABBREVIATIONS

2,4-D	2,4-Dichlorophenoxyacetid acid
<i>A</i>	Final absorbance (nm)
<i>A<sub>o</sub></i>	Initial absorbance (nm)
Abs	Absorbance (nm)
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AQ	Anthraquinone
C	Carbon
<i>C<sub>e</sub></i>	Concentration of solute in the solution at equilibrium (mg/L)
<i>C<sub>i</sub></i>	Initial concentration of solute in the solution (mg/L)
CH <sub>3</sub> COONa	Sodium acetate
CME	Controlled medium exchange
<i>D</i>	Number of variables in the isotherm
DCW	dry cell weight
FTC	Ferric thiocyanate method
FCW	Fresh cell weight
x <i>g</i>	Times gravity
G	Intermediary medium
GCMS	Gas chromatography-mass spectrometry
H	Hydrogen
H <sup>+</sup>	Hydrogen ion
H <sup>+</sup> -ATPase	H <sup>+</sup> -translocating adenosine triphosphatase



$H^+$ -PPiase	$H^+$ -translocating pyrophosphatase
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
$H_2SO_4$	Sulfuric acid
$K^+$	Potassium ion
$K_F$	Equilibrium constants of Freundlich equation (L/mg)
$K_L$	Equilibrium constants of Langmuir equation (L/mg)
$K_R$	Equilibrium constants for Redlich-Petersen equation (L/mg)
LCMS	Liquid chromatography-mass spectrometry
log	Logarithm
M	Molarity (in solution)
M	Maintenance medium (in cell culture)
MeOH	Methanol
MS	Murashige and Skoog
$n$	Constants of Freundlich equation (dimensionless)
N	Normality
$N$	Number of reading
n/a	Not available
Na	Sodium
NAA	$\alpha$ -Naphthaleneacetic acid
NaOH	Sodium hydroxide
ND	Not detectable
$NH_4^+$	Ammonium ion





NH <sub>4</sub> OH	Ammonium hydroxide
NO <sub>3</sub> <sup>-</sup>	Nitrate ion
NPAAs	Nonprotein amino acids
O <sub>2</sub>	Oxygen
OH <sup>-</sup>	Hydroxide ion
P	Production medium
P <sub>i</sub>	Phosphate
PCTC	Plant cell and tissue culture
pH	Potential of the hydrogen ion
pK <sub>a</sub>	Negative logarithm of the acid dissociation constant
PS-DVB	Polystyrene-divinylbenzene
<i>q<sub>e</sub></i>	Amount of solute adsorbed per unit weight of adsorbent at equilibrium (mg/mg)
<i>Q<sub>o</sub></i>	Theoretical monolayer saturation capacity (L/mg)
R <sup>2</sup>	Regression correlation coefficient
SAS	Statistical analysis system
SE	Standard error
SP	Secondary products
SP <sup>+</sup>	Protonated secondary products
SSE	Sum of the errors square
t <sub>R</sub>	Retention time
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
<i>V<sub>e</sub></i>	Final liquid volume at equilibrium (L)

