UNIVERSITI PUTRA MALAYSIA

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

CHIANG LIM

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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

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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 fulfilment of the requirement for the degree of Master of Science

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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-ω-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- ω-metil eter dikesan dari resin XAD-4. Beberapa komponen yang tidak dikenali juga kerap dikesan dari ekstrak AQ.

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I certify that an Examination Committee has met on **8th 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:

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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:

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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.

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CHIANG LIM

Date: before 27 APRIL 2007

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2,4-D	2,4-Dichlorophenoxyacetid acid
A	Final absorbance (nm)
A_o	Initial absorbance (nm)
Abs	Absorbance (nm)
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AQ	Anthraquinone
C	Carbon
C_e	Concentration of solute in the solution at equilibrium (mg/L)
C_i	Initial concentration of solute in the solution (mg/L)
CH_3COONa	Sodium acetate
CME	Controlled medium exchange
D	Number of variables in the isotherm
DCW	dry cell weight
FTC	Ferric thyocyanate method
FCW	Fresh cell weight
x g	Times gravity
G	Intermediary medium
GCMS	Gas chromatography-mass spectrometry
H	Hydrogen
H^+	Hydrogen ion
H^+-ATPase	H^+-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	α-Napthaleneacetic acid
NaOH	Sodium hydroxide
ND	Not detectable
NH_4^+	Ammonium ion

NH_4OH	Ammonium hydroxide
NO_3^-	Nitrate ion
NPAAs	Nonprotein amino acids
O_2	Oxygen
OH^-	Hydroxide ion
P	Production medium
P_i	Phosphate
PCTC	Plant cell and tissue culture
pH	Potential of the hydrogen ion
pK_a	Negative logarithm of the acid dissociation constant
PS-DVB	Polystyrene-divinylbenzene
q_e	Amount of solute adsorbed per unit weight of adsorbent at equilibrium (mg/mg)
Q_o	Theoretical monolayer saturation capacity (L/mg)
R^2	Regression correlation coefficient
SAS	Statistical analysis system
SE	Standard error
SP	Secondary products
SP^+	Protonated secondary products
SSE	Sum of the errors square
t_R	Retention time
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
V_e	Final liquid volume at equilibrium (L)