

**IDENTIFICATION OF MORPHOLOGY,
GROWTH KINETIC AND SECONDARY
METABOLITES SYNTHESIZED IN GREEN
FRESHWATER MICROALGAE (*COCCOMYXA
DISPAR*)**

JOEY LEW HUI LIN

UNIVERSITI SAINS MALAYSIA

JUNE 2020



**PUSAT PENGAJIAN
TEKNOLOGI INDUSTRI
UNIVERSITI SAINS MALAYSIA**

**BORANG PENYERAHAN DISERTASI
MUTAKHIR SATU (1) NASKAH**

Nama penyelia: Dr. Mohamad Hafizi Abu Bakar

Bahagian: Bioprocess Technology

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh
Cik Joey Lew Hui Lin
mengenai disertasinya sebagaimana yang dipersetujui oleh Panel Pemeriksa di Viva
Vocanya.

2. Saya ingin mengesahkan bahawa saya berpuashati dengan pembetulan/pindaan
yang dilaksanakan oleh calon.

Sekian, terima kasih.

20/07/2020

(Tandatangan dan cop)

Tarikh

DR. MOHAMAD HAFIZI ABU BAKAR
SENIOR LECTURER
SCHOOL OF INDUSTRIAL TECHNOLOGY
UNIVERSITI SAINS MALAYSIA



**IDENTIFICATION OF MORPHOLOGY,
GROWTH KINETIC AND SECONDARY
METABOLITES SYNTHESIZED IN GREEN
FRESHWATER MICROALGAE (*COCCOMYXA
DISPAR*)**

by

JOEY LEW HUI LIN

A dissertation submitted in the partial fulfillment of the requirements for the degree
of Bachelor of Technology (B.Tech) in the field of Bioprocess Technology

School of Industrial Technology

Universiti Sains Malaysia

June 2020

DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.



JOEY LEW HUI LIN

JUNE 2020

ACKNOWLEDGEMENTS

First and foremost, I would like to show my sincere gratitude to my final year project supervisor, Dr. Mohamad Hafizi Abu Bakar for his support and guidance throughout the project. His knowledge and advices had helped me a lot in completing this project.

Besides, special thanks to my co-supervisor, Dr. Mohd Asyraf Kassim for providing me microalgae *Coccomyxa dispar* and taught me a lot about the cultivation of microalgae along this research. A special mention for Bioprocess Division lab assistants, Mr. Azmaizan bin Yaakub and Mrs. Najmah binti Hamid for the technical support in this research.

Furthermore, I would like to take this opportunity to express my appreciativeness to School of Industrial Technology for giving me the chance to use the available facilities and equipments throughout this research project. Special thanks to postgraduate students, Mr. Tan Kean Meng, Mr. Mohamad Shamil Faris Bin Mohamad Khalid, Ms. Nor Shafiqah Binti Nor Shahril and Mr. Ng Wai Chun for their support and guidance throughout this research.

Finally, I would like to thank to my supporting coursemate and friends who supported and encouraged me during my research study. Also, a special thanks to my lovely family for their love, blessing and support along this research.

JOEY LEW HUI LIN

June 2020

TABLE OF CONTENTS

	Page
Acknowledgements	iii
Table of Contents	iv
List of Tables	vii
List of Figures	viii
List of Symbols and Abbreviations	ix
Abstrak	xii
Abstract	xiv
CHAPTER 1 INTRODUCTION	
1.1 Research background	1
1.2 Problem statement	3
1.3 Research objectives	5
CHAPTER 2 LITERATURE REVIEW	
2.1 General overview of microalgae	6
2.2 Cell structural and morphological features of microalgae	8
2.3 Cell division	10
2.4 Cultivation mode	11
2.5 Microalgae growth phase	12
2.6 Bioactive metabolites	14
2.6.1 Primary metabolites	
2.6.1a Proteins	16
2.6.1b Carbohydrates	16
2.6.1c Lipids	17
2.6.2 Secondary metabolites	

2.6.2a Carotenoids	18
β-Carotene	19
Astaxanthin	20
Lutein and zeaxanthin	20
Violaxanthin	21
Fucoxanthin	22
2.6.2b Phenolic compounds	22
2.6.2c Phytosterol	23
2.6.2d Phycobiliprotein	24
2.7 <i>Coccomyxa</i> sp.	24
CHAPTER 3 MATERIALS AND METHODS	
3.1 Overall experimental design	27
3.2 Apparatus and equipment	28
3.3 Chemical and reagents	28
3.4 Cultivation of <i>Coccomyxa dispar</i>	28
3.5 Cell morphology observation	29
3.6 Cell recovery	30
3.7 Measurement of cell growth kinetic	30
3.8 Cell disruption	31
3.9 Solvent extraction	31
3.10 GC-FID	
3.10.1 Algae sample solution preparation	32
3.10.2 GC-FID analysis	33
3.11 Statistical analysis	33
CHAPTER 4 RESULTS AND DISCUSSION	

4.1 Cell morphology	34
4.2 Cell growth kinetic measurement	36
4.3 Effects of different solvents on extraction yield	41
4.4 Secondary metabolites analysis by GC-FID	43
CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH	
5.1 Conclusions	51
5.2 Recommendations for future research	52
REFERENCES	54
APPENDICES	65

LIST OF TABLES

Table Caption	Page
2.1 Examples of microalgae in different morphological form	9
4.1 Doubling time, maximum biomass concentration and productivity biomass of <i>Coccomyxa dispar</i>	41

LIST OF FIGURES

Figure Caption	Page
2.1 General schematic growth curve of a microalgae batch culture system (a) and corresponding variations of cell population growth rate (b).	14
3.1 Overall experimental design of cell morphology, growth kinetic and secondary metabolites analysis of <i>Coccomyxa dispar</i> microalgae.	27
4.1 Morphology of <i>Coccomyxa dispar</i> at x 1000 magnification under Olympus CX41 upright microscope.	35
4.2 Graph of cell dry weight against cultivation time of <i>Coccomyxa dispar</i> (growth curve).	39
4.3 Graph of growth rate against cultivation time of <i>Coccomyxa dispar</i> .	39
4.4 Graph of extract yield percentage of each solvent (hexane, ethyl acetate and methanol).	43
4.5 GC-FID chromatogram of <i>Coccomyxa dispar</i> extract isolated using hexane.	48
4.6 GC-FID chromatogram of <i>Coccomyxa dispar</i> extract isolated using ethyl acetate.	49
4.7 GC-FID chromatogram of <i>Coccomyxa dispar</i> extract isolated using methanol.	50

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Caption
+	Plus
-	Minus
±	Plus minus
%	Percentage
β	Beta
°C	Degree Celsius
μ	Micro
<	Less than
Abbreviation	Caption
Abs	Absorbance
atm	Atmosphere
BBM	Bold basal medium
C	Carbon
COX-2	Cyclooxygenase-2
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EPA	Eicosapentaenoic acid
FID	Flame ionization detector
g	Gram
GC	Gas chromatography
h	Hour
H	Hydrogen

H ₂ O	Water
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IκBα	Inhibitor of NFκB, alpha
L	Litre
LPS	Lipopolysaccharides
lx	Lux
M	Molar
min	Minute
mL	Millilitre
μg	Microgram
μg	Micrometer
μL	Microlitre
MS	Mass spectrometry
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
nm	Nanometer
NO	Nitric oxide
OD	Optical density
PGE ₂	Prostaglandin E ₂
pH	Power of hydrogen
PUFA	Polyunsaturated fatty acid
rpm	Revolutions per minute
SD	Standard deviation
Sp.	Species

TNF- α	Tumor necrosis factor alpha
UV	Ultraviolet
Vis	Visible
Wt.	Weight
X	Biomass concentration

**IDENTIFIKASI MORFOLOGI, KINETIK PERTUMBUHAN DAN
METABOLIT SEKUNDER YANG DIHASILKAN OLEH MIKROALGA
HIJAU JENIS AIR TAWAR (*COCCOMYXA DISPAR*)**

ABSTRAK

Mikroalga merupakan sumber yang signifikan untuk menghasilkan pelbagai bioaktif metabolit. Mikroalga dapat diperbaharui, mudah untuk dikultur, mempunyai waktu generasi yang sangat cepat dan boleh menyelesaikan masalah kekurangan tanah yang disebabkan oleh kegunaan sumber tumbuhan. Metabolit yang dihasilkan boleh digunakan sebagai produk suplemen kesihatan dan farmaseutikal. Projek ini bertujuan untuk menganalisis metabolit yang dihasilkan oleh *Coccomyxa dispar* yang dikultur dalam BBM dalam pH 3.3. Mikroalga dikultur dalam suhu bilik dengan intensiti cahaya 1500 lux dan diudarakan selama 24 jam. Morfologi mikroalga diidentifikasi dengan menggunakan mikroskop cahaya. Pertumbuhan mikroalga selama 15 hari telah diketahui dengan mengukur kerapatan optik menggunakan cara spektrofotometri. Metabolit sekunder telah diasingkan menggunakan pelarut heksana, etil asetat dan methanol, dan dianalisis dengan GC-FID. *Coccomyxa dispar* merupakan mikroalga uniseluler yang berbentuk oval, berwarna hijau dan mengandungi kloroplas parietal tunggal. *Coccomyxa dispar* mencapai pertumbuhan maksimum pada hari ke-4, iaitu 0.1647 hari^{-1} dan mencapai fasa penurunan pertumbuhan pada hari ke-5. Penghasilan metabolit sekunder biasanya berlaku pada fasa ini disebabkan oleh kehabisan nutrien. *Coccomyxa dispar* memiliki biojisim maksimum 0.2680 g L^{-1} dan produksi biojisim $0.0155 \text{ g L}^{-1} \text{ hari}^{-1}$. Pelarut metanol memiliki hasil ekstrak tertinggi iaitu 31.13 %, kedua ialah hexane (10.47 %) dan diikuti oleh ethyl acetate (6.55 %). Hasil penelitian menunjukkan bahawa hasil ekstrak mikroalga berbeza nyata berbanding dengan

tiga pelarut ($p < 0.05$). Dengan melakukan GC-FID, lutein, zeaxanthin, violaxanthin, EPA, β -sitosterol dan stigmasterol telah diidentifikasi dan dibandingkan dengan kromatogram dalam kajian sebelumnya. Walaupun bioaktif metabolit yang diekstrak dari *Coccomyxa dispar* masih rendah, hasil metabolit juga mungkin ditingkatkan dengan cara-cara yang lain.

**IDENTIFICATION OF MORPHOLOGY, GROWTH KINETIC AND
SECONDARY METABOLITES SYNTHESIZED BY GREEN FRESHWATER
MICROALGAE (*COCCOMYXA DISPAR*)**

ABSTRACT

Microalgae are considered a promising source of organism that can be cultured and isolated to obtain a range of functional metabolites. They are renewable, easily to be cultured and have short generation time which overcome land limitation problem caused by utilization of plant resources. Their metabolites can be used as a source of natural ingredients in pharmaceutical and nutraceutical products. This study aims to analyse potential metabolites in freshwater microalgae, *Coccomyxa dispar* in BBM medium with pH 3.3. The cultivation was carried out in room temperature with light intensity 1500 lux and aerated for 24 hours. Morphology of microalgae has been identified under light microscope. The growth of the microalgae cell within these 15 days was investigated by determining the optical density of microalgae using spectrophotometer. Secondary metabolites were isolated using hexane, ethyl acetate and methanol and were analysed using GC-FID. *Coccomyxa dispar* was a green unicellular microalga which had elongated oval cells and contained single parietal chloroplast as observed under microscope. *Coccomyxa dispar* reached its maximum growth rate at day 4, which was 0.1647 day^{-1} and entered retardation phase at day 5 where production of secondary metabolites took place due to the limiting nutrient. The maximum biomass concentration after 15 days obtained was 0.2680 g L^{-1} and the overall biomass productivity was $0.0155 \text{ g L}^{-1} \text{ day}^{-1}$. Among the solvents tested, methanol showed the highest extraction yield which was 31.13 %, followed by hexane (10.47 %) and ethyl acetate (6.55 %). The extraction yield of microalgae was significantly difference among the three solvents,

at $p < 0.05$. Using GC-FID, lutein, zeaxanthin, violaxanthin, EPA, β -sitosterol and stigmasterol were identified based on matching the retention times of the chromatogram in previous study. Although the levels of secondary metabolites present in *Coccomyxa dispar* are still low, it does not eliminate the possibility of being increased.