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

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Sekian, terima kasih.

(Tandatangan dan cop)

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by

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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

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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.

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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_2O	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	Visble
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 dihasil 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 dianalisiskan 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 g L^{-1} $hari^{-1}$. 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 g L^{-1} 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.