

**THE CULTIVATION OF IMMOBILIZED  
*Chlorella vulgaris* IN DIFFERENT CULTURE  
MEDIUM FOR BIODIESEL PRODUCTION**

**NUR HANANI BINTI RUSHAN**

**MASTER OF SCIENCE**

**UNIVERSITI MALAYSIA PAHANG**



### **SUPERVISOR'S DECLARATION**

We hereby declare that we have checked this thesis and, in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.

---

(Supervisor's Signature)

Full Name : TS. DR. NUR HIDAYAH BINTI MAT YASIN

Position : SENIOR LECTURER

Date : 3/5/2021

---

(Co-supervisor's Signature)

Full Name : TS. DR. FARHAN BINTI MOHD SAID

Position : SENIOR LECTURER

Date : 4/5/2021



### STUDENT'S DECLARATION

I hereby declare that the work in this 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 Universiti Malaysia Pahang or any other institutions.

A handwritten signature in black ink, appearing to read 'Nur Hanani Binti Rushan', is placed over a horizontal line.

(Student's Signature)

Full Name : NUR HANANI BINTI RUSHAN

ID Number : MKC17017

Date : 3/5/2021

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**NUR HANANI BINTI RUSHAN**

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## ABSTRAK

Mikroalga dijadikan sebagai sumber alternatif dan bahan menghasilkan minyak kerana struktur uniselular dan kecekapan fotosintesis yang tinggi. Dalam eksperimen ini, *Chlorella vulgaris* dipilih sebagai mikroalga kerana spesis ini boleh menghasilkan minyak yang tinggi dalam pemprosesan biodiesel. Immobilisasi adalah salah satu kaedah penuaian yang digunakan dalam kajian ini kerana kurang menggunakan tenaga dan lebih mudah dikendalikan. Sebelum ini, natrium alginat (SA) adalah sistem matrik yang selalu digunakan untuk memerangkap mikroalga dalam proses kultur. Walaubagaimanapun, SA mempunyai beberapa kekurangan seperti manik mudah pecah yang menyebabkan kehilangan sel mikroalga. Di samping itu, keperluan nutrien yang diperlukan adalah salah satu faktor penting yang perlu dititikberatkan untuk meningkatkan pengeluaran minyak. Oleh itu, gabungan sistem matrik telah diperkenalkan dalam kajian ini untuk meningkatkan pengeluaran minyak. Dalam kajian ini, SA bertindak sebagai kawalan manakala lima sistem matrik yang berbeza digabungkan dengan SA seperti kitosan (SA+CT), karagenan (SA+CR), gelatin (SA+GT), kalsium alginat (SA+CA) dan natriumkarboksimetil selulosa (SA+CMC). Objektif pertama dalam kajian ini adalah mengkaji kultur medium yang berbeza untuk *C. vulgaris* dengan menggunakan *Medium Bold's Basal Medium* (BBM), *Blue-Green Medium* (BG11) dan *Jaworski's medium* (JM). Manakala objektif kedua adalah untuk menilai jumlah pengeluaran minyak dari immobilisasi *C. vulgaris* menggunakan sistem matrik yang berbeza dan nisbah yang berbeza iaitu 0.3:1, 1:1 and 2:1 untuk pengeluaran biodiesel. Analisis metil ester asid lemak yang diekstrak dari mikroalga air tawar adalah objektif ketiga manakala penentuan kinetik dan termodinamik parameter seperti kadar tindak balas pemalar dan tenaga pengaktifan menggunakan persamaan Arrhenius merupakan objektif keempat dalam kajian ini. Pada awalnya, mikroalga dikultur, dituai dan diekstrak menggunakan kaedah pengekstrakan pelarut untuk menghasilkan minyak, sebelum digunakan dalam proses transesterifikasi dengan menggunakan Spektrofotometer jisim kromatografi gas (GC-MS). Kemudian metil ester asid lemak yang diekstrak dari *C. vulgaris* dianalisa menggunakan spektrometri jisim kromatografi gas (GC-MS). Berdasarkan hasil yang diperolehi, kultur media terbaik adalah BBM yang menunjukkan hasil minyak tertinggi iaitu 27.14% manakala kombinasi sistem matrik SA+GT menunjukkan hasil minyak tertinggi dengan 44.29%. Dalam kajian ini, komponen utama metil ester asid lemak dalam *C. vulgaris* yang diekstrak dari mikroalga menunjukkan potensi yang tinggi untuk penghasilan biodiesel kerana mengandungi asid palmitik (C16:0), asid stearik (C18:0), asid oleik (C18:1), asid linoleik (C18: 2) dan asid linolenik (C18:3). Peratusan asid lemak tepu (C16:0 & C18:0) adalah lebih tinggi daripada asid lemak tak tepu (C18:1, C18:2 & C18:3). Kajian kinetik menunjukkan bahawa nilai tenaga pengaktifan (*Ea*) untuk kinetik pengekstrakan minyak dari mikroalga adalah 26.382 kJ/mol. Entalpi ( $\Delta H$ ) dan entropi ( $\Delta S$ ) menunjukkan nilai positif manakala tenaga Gibbs ( $\Delta G$ ) adalah negatif dan menunjukkan bahawa proses ini bersifat endotermik, proses tidak berpatah balik dan spontan. Hasil kajian ini menunjukkan bahawa BBM lebih bagus dan berkesan dalam proses pengkulturan dan penerapan menggunakan sistem matrik SA+GT kerana ia dapat membentuk struktur baru yang dapat meningkatkan pengeluaran minyak daripada hanya menggunakan matrik tunggal. Tambahan lagi, profil metil ester asid lemak (FAME) menunjukkan potensi yang besar dalam pengeluaran biodiesel.

## ABSTRACT

Microalgae is known as an alternative source and promising feedstock for biodiesel production that can produce oil due to their simple unicellular structure and high photosynthetic efficiency. In this experiment, *Chlorella vulgaris* is selected as microalgae as this species is able to produce high oil for biodiesel processing. Immobilization is one of the harvesting methods employed in this study due to less energy consumed and ease of handling. Previously, the matric system which is sodium alginate (SA) was commonly used to entrap the microalgae in culturing process. However, SA has certain limitation such as bead disruption or bead dissolution that lead to the loss of microalgae cell. In addition, the required nutrient is one of the constraints that need to be overcome to enhance the production of oil. Therefore, the combination of matric system has been developed in this study in order to enhance the production of oil. In the present study, SA acts as a control whereas five different matric systems were combined with SA such as chitosan (SA+CT), carrageenan (SA+CR), gelatine (SA+GT), calcium alginate (SA+CA) and sodium carboxymethylcellulose (SA+CMC). The first objective in this study was to elucidate different culture medium of *C. vulgaris* by using Bold's Basal Medium (BBM), Blue-Green Medium (BG11) and Jaworski's medium (JM) while the second objective was to evaluate the oil production of immobilized *C. vulgaris* using different matric systems at different volumetric ratios of 0.3:1, 1:1 and 2:1 for biodiesel production. Besides, analysing the fatty acid methyl ester which extracted from freshwater microalgae was the third objective while determining kinetic and thermodynamic parameter were exhibited using reaction rate equation and Gibbs energy equation was the fourth objective in this study. The microalgae were first cultivated, harvested and extracted using solvent extraction method to produce oil, prior to use in the transesterification process using Gas chromatography mass spectrophotometer (GC-MS). Then, the fatty acid methyl ester extracted from *C. vulgaris* was analysed using GC-MS. Based on the results obtained, the best culture media was BBM which showed the highest oil yield which is 27.14% whereas the combination of SA+GT as a matric showed the highest oil yield with 44.29%. In this study, the main components of fatty acid methyl ester in the *C. vulgaris* of oil extracted from microalgae showed high potential for biodiesel production as it consisted of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). The percentage of saturated fatty acid (C16:0 & C18:0) were higher than the unsaturated fatty acid (C18:1, C18:2 & C18:3). The kinetic study shows that the value of activation energy (*E<sub>a</sub>*) for the oil extraction kinetics of microalgae biomass was calculated as 26.382 kJ/mol. Both enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) indicate positive value whereas the negative value of Gibbs energy ( $\Delta G$ ) indicates that this process is endothermic, irreversible and spontaneous. The research findings show that the BBM was more effective in culturing process and the applicability of the matric systems of SA+GT made a new structure that improve the oil production than using single matric. Furthermore, the similar fatty acid methyl ester (FAME) profile was showing a huge potential for biodiesel production.

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