BIOMASS, OIL AND FATTY ACIDS PROFILING OF MANGROVE-ISOLATED MICROALGAE UNDER SELECTED CULTIVATION CONDITIONS

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Institute of Marine Biotechnology
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Dedicated this thesis to:

My supervisor, Associate Professor Cha Thye San, Ph.D.

My co-supervisor, Professor Aziz Bin Ahmad, Ph.D.

My beloved parents and siblings and lastly my colleagues

For all their dedication, sacrifice and endless love.

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Master of Science

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Main Supervisor : **Associate Professor Cha Thye San, Ph.D.**

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Microalgae have been recognized for their substantial amount of oil content along with high long-chain marketable polyunsaturated fatty acids (PUFAs) such as omega-3 and -6 fatty acids. However, indigenous microalgae in Malaysia are mostly untapped resources for lipid assessment due to lack of research conducted, in particular the species from mangrove environment. Therefore, eight new mangrove-isolated microalgae species were successfully described through morphological characterization and molecular identification with 18S rDNA. The identified strains include the species from the genus of *Chlorella, Desmodesmus, Chlamydomonas and Scenedesmus*. In order to screen these species for potential oil and fatty acid productions, a small-scale cultivation system and an optimized oil extraction protocol were established. This enable cost-saving in term of reduce chemicals usage by 50% and increase efficiency from time-saving. The eight newly characterized species together with two model species (*Chlorella vulgaris* UMT-M1 and *Messastrum*

gracile SE-MC4) were subjected to nitrate-replete and nitrate-deficient cultivation conditions and its growth, biomass, oil content and fatty acid composition were determined. Result revealed that the microalgae could be categorized into high cell growth with high biomass (Group I), moderate cell growth with wider range of biomass (Group II), and degrading cell growth with low biomass (Group III). Interestingly, five strains (UMT-M1, SE-MC4, KS-2, UMT-B13 and UMT-B15) were regarded as oleaginous microalgae as they produced more than 20% (in between 23.5-37.0%) of total oil content under the studied conditions. Furthermore, fatty acid profiling revealed that six strains (UMT-B8, -B13, -B14 and -B15, UMT-M1 and KS-2) were high saturated fatty acid (SFA) producers (or Group H-SFA) with total SFA ranging between 52.4-97.1%. Three strains (SE-MC4, UMT-B10, -B20) were high SFA and monounsaturated fatty acid (MUFA) producers (or Group H-SFA: MUFA) with each constituted more than 30% while one high producer (UMT-B11) for SFA and polyunsaturated fatty acids (or Group H-SFA: PUFA) with each constituted more than 30%. In addition, the influence of different growth phases and culture transition phase on the selected species, *M. gracile* SE-MC4 were also evaluated. Results showed that the biomass and total oil content increased in parallel to the cultivation day and achieved maximum at late stationary phase (day 8). This species accumulated high amount of C18:1 (32.4%) at late stationary phase and dropped instantaneously to 13% during the culture transition into Stage II cultivation. This was in contrast to the accumulation of C18:3n3, where its content spiked up from a lowest to a maximum of 23.0%. These demonstrated that the storage C18:1 pool in the late stationary phase was immediately used as a substrate for the synthesis of C18:3n3 which could play an important role during the early phase of microalgae growth. The mangrove microalgae can be looked up as a new prospective of lipids and fatty acid sources for various different applications. Understanding the biochemical properties of interest under specific stage of growth is important for future fundamental study to formulate further experiment that could increase the lipid content without compromising its biomass.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENCIRIAN BIOJISIM, MINYAK DAN PROFIL ASID LEMAK DARIPADA MIKROALGA PAYA BAKAU DIDALAM KEADAAN PENGKULTURAN TERPILIH

NUR SYAZANA BINTI FAUZI

2021

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Mikroalga telah diiktiraf kerana jumlah kandungan minyak yang tinggi bersama-sama dengan asid lemak poli-tak tepu (PUFAs) yang tinggi seperti omega-3 dan -6. Walau bagaimanapun, mikroalga dari Malaysia merupakan sumber yang jarang diterokai untuk penghasilan lemak kerana kekurangan penyelidikan yang dijalankan, khususnya spesis dari persekitaran paya bakau. Oleh itu, lapan spesis mikroalga paya bakau berjaya dikelaskan melalui pencirian morfologi dan pengecaman molekul dengan 18S rDNA. Strain yang telah dikelaskan termasuk spesis dari genus *Chlorella*, *Desmodesmus*, *Chlamydomonas* dan *Scenedesmus*. Untuk menyaring spesis yang berpotensi dalam pengeluaran minyak dan asid lemak, sistem pengkulturan berskala kecil dan protokol ekstraksi minyak yang optimum telah berjaya dihasilkan dalam kajian ini. Kaedah-kaedah ini membolehkan penjimatan kos dari segi mengurangkan penggunaan bahan kimia sebanyak 50% dan meningkatkan kecekapan pengekstrakan minyak dari segi penjimatan masa. Lapan spesis yang baru dikelaskan bersama-sama dengan dua spesis model (*Chlorella vulgaris* UMT-M1 dan *Messastrum gracile* SE-

MC4) telah dikultur dalam keadaan nitrat-penuh dan nitrat-terhad, dan pertumbuhannya, biojisim, kandungan minyak dan komposisi asid lemak telah ditentukan. Hasil kajian menunjukkan bahawa mikroalga boleh dikategorikan kepada pertumbuhan sel yang tinggi dengan biojisim tinggi (Kumpulan I), pertumbuhan sel sederhana dengan biojisim berbeza (Kumpulan II), dan pertumbuhan sel yang rendah dengan biojisim rendah (Kumpulan III). Menariknya, lima strain (UMT-M1, SE-MC4, KS-2, UMT-B13 dan UMT-B15) diketegorikan sebagai mikroalga pengeluar minyak kerana dapat menghasilkan lebih daripada 20% (di antara 23.5-37.0%) kandungan minyak di bawah keadaan kultur yang dikaji. Selain itu, profil asid lemak mendedahkan bahawa enam strain (UMT-B8, -B13, -B14 dan -B15, UMT-M1 dan KS-2) adalah pengeluar asid lemak tepu tinggi (atau Kumpulan H-SFA) dengan jumlah SFA di antara 52.4 -97.1%. Tiga strain (SE-MC4, UMT-B10, -B20) adalah pengeluar tinggi asid lemak tepu (SFA) dan asid lemak mono-tak tepu (MUFA) (atau Kumpulan H-SFA: MUFA) dengan masing-masing membentuk lebih daripada 30%, manakala satu strain (UMT-B11) pengeluar tinggi asid lemak tepu (SFA) dan asid lemak poli-tak tepu (PUFA) (atau Kumpulan H-SFA: PUFA) dengan masing-masing membentuk lebih daripada 30%. Di samping itu, pengaruh fasa pertumbuhan yang berbeza dan fasa peralihan kultur terhadap spesis terpilih, *M. gracile* SE-MC4 juga telah dikaji. Keputusan menunjukkan bahawa biojisim dan jumlah kandungan minyak meningkat selari dengan hari pengkulturan dan mencapai jumlah maksimum pada fasa lewat pegun (hari ke-8). Spesis ini telah mengumpul jumlah C18:1 (32.4%) yang tinggi pada fasa lewat pegun dan telah turun dengan serta-merta kepada 13% semasa peralihan kultur ke Tahap II pengkulturan. Ini bertentangan dengan pengumpulan C18:3n3, di mana kandungannya meningkat daripada terendah hingga ke maksimum 23.0%. Ini menunjukkan bahawa penyimpanan C18:1 yang banyak dalam fasa lewat pegun segera digunakan sebagai substrat untuk menghasilkan C18:3n3 yang mana mempunyai peranan penting semasa fasa pertumbuhan awal mikroalga. Mikroalga paya bakau dapat dilihat sebagai prospek baru sumber lipid dan asid lemak untuk pelbagai aplikasi yang berbeza. Memahami sifat biokimia dalam tahap pertumbuhan tertentu adalah penting bagi kajian asas masa depan untuk merumuskan eksperimen selanjutnya dalam meningkatkan kandungan lipid tanpa menjejaskan jisimnya.