CULTIVATION OF MICROALGAE *NANNOCHLOROPSIS* SP. IN PALM OIL MILL EFFLUENT FOR BIODIESEL PRODUCTION

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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.

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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.

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ABSTRAK

Penggunaan sumber fosil secara meluas mengakibatkan kekurangan bahan bakar fosil dengan cepat. Biodiesel merupakan sumber tenaga alternatif yang boleh diperbaharui dan dapat mengurangkan pergantungan pada bahan bakar fosil. Penjanaan biodiesel generasi terdahulu tidak ideal sebagai pengganti bahan bakar fosil kerana persaingan tanah untuk penanaman sumber makanan manusia. Untuk mengatasi persaingan tanah ini, biodiesel dihasilkan daripada sumber mikroalga. Walau bagaimanapun, penanaman mikroalga adalah mahal kerana memerlukan sumber nutrien dan air dalam jumlah besar. Akibatnya, efluen kilang kelapa sawit (POME), yang mengandungi kandungan nutrien yang banyak digunakan sebagai medium permakanan alternatif untuk mengurangkan kos pertumbuhan mikroalga. Dalam kajian ini, mikroalga dari Teluk Cempedak, Kuantan yang mempunyai kandungan lipid yang tinggi diasing dan dikultur untuk penghasilan biodiesel. Enam jenis mikroalga disaring dan dikenal pasti dalam pemeriksaan awal dengan kajian morfologi. Namun, mikroalga hijau *Nannochloropsis* sp. telah dipilih kerana memiliki kadar pertumbuhan yang tinggi dan kandungan lipid yang banyak. Faktor yang mempengaruhi penghasilan lipid intraselular seperti kesan kepekatan POME, jangkamasa pendedahan kepada cahaya dan panjang gelombang cahaya LED dikaji untuk menentukan faktor optimum bagi pertumbuhan dan penghasilan lipid *Nannochloropsis* sp. Pengkulturan *Nannochloropsis* sp. secara skala besar diikuti dengan kaedah pengekstrakan Soxhlet telah menghasilkan kandungan lipid sebanyak 61.5%. Lipid yang diekstrak kemudian ditransesterifikasikan dengan metanol dan kalium hidroksida menghasilkan metil ester (biodiesel) dalam 1.5 jam. Analisis kromatografi lapisan nipis (TLC) dilakukan untuk memastikan penukaran lipid *Nannochloropsis* sp. ke biodiesel. Pembentukan metil ester asid lemak yang diperoleh adalah sebanyak 80.24%. Kromatogram gas kromatografi spektrometri jisim (GS-MS) menggambarkan metil ester dari *Nannochloropsis* sp. mengandungi kandungan asid oleik ($C_{18:1}$) dan asid palmitik ($C_{16:0}$) yang tinggi, masing-masing sebanyak 66.13% dan 12.38%. Lipid *Nannochloropsis* sp. berpotensi untuk digunakan dalam industri biodiesel kerana komposisi asid lemak dan lipid yang tinggi.

ABSTRACT

Extensive usage of fossil reserves resulted in rapid fossil fuel depletion. Biodiesel is one of the renewable energy alternatives designed to reduce reliance on fossil fuels. The generation of biodiesel from edible and non-edible crops is not identified as an ideal substitute to fossil fuels due to the competition for limited cultivable land proposed to cultivate crops for human consumption. To overcome the drawback, biodiesel is derived from the cultivation of microalgae. However, the cultivation of microalgae is costly as they require nutrients and water in a large amount. As a result, palm oil mill effluent (POME), which contains a large number of nutrients required for microalgae growth, is used as an alternative nutrition medium for microalgae cultivation while treating wastewater. In this study, attempts have been made to isolate and mass cultivate high lipid content microalgae from Teluk Cempedak, Kuantan coast, for biodiesel production. Among the collected samples, six microalgae were screened upon preliminary screening for morphological studies however green microalgae *Nannochloropsis* sp. was identified to be the most suitable microalgae with a high growth rate and abundant lipid content. Culture factors influencing the intracellular lipid body were investigated. The effect of different POME concentrations, photoperiod regimes and light-emitting diode (LED) light wavelengths were examined to determine the optimum factor for *Nannochloropsis* sp. growth and lipid enhancement. The mass cultivation under combined optimized culture factors of *Nannochloropsis* sp. followed by the Soxhlet extraction method yielded a lipid content of 61.5%. The extracted lipid was then transesterified with methanol to produce methyl esters (biodiesel) in 1.5 h, where potassium hydroxide (KOH) was used as a homogenous catalyst. Thin-layer chromatography (TLC) was done to ensure the conversion of *Nannochloropsis* sp. oil to biodiesel. The highest fatty acid methyl ester (FAME) formation from *Nannochlropsis* sp. was 80.24%. The output of gas chromatography- mass spectrometry (GC-MS) analysis proves that FAME comprises of high amount of oleic acid ($C_{18:1}$) 66.13% and palmitic acid ($C_{16:0}$) 12.38% respectively. *Nannochloropsis* sp. is a promising candidate for biodiesel production due to its composition of fatty acids and higher lipid content.

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