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GROWTH RATE, LIPID CONTENT AND FATTY ACID PROFILES OF MARINE AND FRESHWATER DIATOMS IN MALAYSIA

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By

ABDULFATTAH MOHAMED LANDELSI ELFITURI

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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February 2018

Chairman : Md. Aminur Rahman, PhD
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Diatoms are photosynthetic organisms that can produce lipids in large amounts and within short periods of time, and these can be processed into both biofuels and other useful commercial products. Due to this reason, diatoms are considered as a potential source of renewable energy; and one of the most important decisions in obtaining oil from microalgae is the choice of species. The current study focuses on six species of marine and freshwater diatoms, in terms of their growth rates, lipid, protein, carbohydrate and fatty acid contents. Freshwater diatoms (*Sellaphora* sp., *Nitzschia palea*, *Craticula cuspidata*) were isolated from Kajang pond, Selangor, Malaysia and marine diatoms (*Nitzschia sigma*, *Nitzschia* sp. and Chaetoceros calcitrans) from coastal area of Pangkor, Perak, Malaysia. Diatoms were identified by electron microscope and 18s rDNA. Among the diatom species studied, *C. calcitrans*, *Nitzschia* sp. and *N. sigma* (marine diatom) had significantly higher ($p < 0.05$) specific growth rate with 0.55, 0.51 and 0.33 (μ day^{-1}) respectively, while freshwater diatoms (*Sellaphora* sp., *N. palea*, *C. cuspidata*) grew slower with specific growth rates 0.14, 0.22 and 0.25 (μ day^{-1}). In addition, biomass of marine diatoms was (220,223 and 263 mg/L) higher than freshwater diatoms (90, 113 and 127 mg/L DW). Furthermore, the result of total lipid showed a significant difference ($p < 0.05$) between the mean values of marine and freshwater species. The marine diatoms species had higher lipid content (18.7 to 28 % DW) than freshwater diatoms (13 to 16 % DW). *Nitzschia* sp. had the highest lipid content, while *C. cuspidata* had the lowest lipid content. Also fatty acid showed significant difference ($p < 0.05$) in omega-3 (PUFAn-3). Where, marine diatoms had higher yield of Omega-3 than freshwater diatoms. On the other hand, total protein and carbohydrate were similar ($p > 0.05$) in diatoms, except *Nitzschia* sp. and *C. cuspidata* showed significant difference ($p < 0.05$) in total protein, and carbohydrate.

Overall, in respects of growth rate, biomass, total lipid content and quality of fatty acid, *Nitzschia* sp. (marine diatom) in current study performed better than other species which makes it a good source for lipid (28% DW), protein (32% DW) and omega-3 (21.77 mg/g DW) and can use it as food in aquaculture and as source of biofuels.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KADAR PERTUMBUHAN, KANDUNGAN LIPID DAN PROFIL ASID LEMAK BAGI DIATOM MARIN DAN AIR TAWAR DI MALAYSIA

Oleh

ABDULFATTAH MOHAMED LANDELSI ELFITURI

Februari 2018

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Diatom adalah organisma fotosintetik yang dapat menghasilkan lipid dalam jumlah yang banyak dan dalam masa yang singkat, hal ini membolehkan ianya diproses menjadi bahan bakar bio dan produk komersil lain yang berguna. Oleh sebab itu, diatom dianggap sebagai sumber tenaga yang boleh diperbaharui. Salah satu aspek terpenting dalam mendapatkan minyak dari mikroalga adalah pemilihan spesies. Kajian semasa memberi tumpuan kepada perbandingan antara enam spesies diatom air laut dan air tawar, dari segi kadar pertumbuhan, kandungan lipid, protein, karbohidrat dan asid lemak. Diatom air tawar (*Sellaphora* sp., *Nitzschia palea*, *Craticula* cuspidata) diperoleh dari kolam di Kajang, Selangor, Malaysia dan diatom air laut (*Nitzschia sigma*, *Nitzschia* sp. dan *Chaetoceros calcitrans*) diambil dari kawasan pesisir laut di Pangkor, Perak, Malaysia. Diatom dikenal pasti menggunakan mikroskop elektron dan 18s rDNA. Antara spesies diatom yang dikaji, *C. calcitrans*, *Nitzschia* sp. dan *N. sigma* (diatom air laut) mempunyai kadar pertumbuhan spesifik yang signifikan ($p < 0.05$) dengan kadar masing-masing 0.55, 0.51 dan 0.33 (μ hari^{-1}), walaubagaimanapun kadar pertumbuhan spesifik diatom air tawar (*Sellaphora* sp., *N. palea*, *C. cuspidata*) lebih perlahan dengan kadar pertumbuhan 0.14, 0.22 dan 0.25 (μ hari^{-1}). Di samping itu, biojisim diatom laut adalah (220,223 dan 263 mg/L) lebih tinggi daripada diatom air tawar (90, 113 dan 127 mg/L DW). Tambahan pula, hasil lipid keseluruhan menunjukkan perbezaan yang signifikan ($p < 0.05$) antara nilai min spesies air laut dan air tawar. Spesies diatom air laut mempunyai kandungan lipid yang lebih tinggi (18.7 hingga 28% DW) daripada diatom air tawar (13 hingga 16% DW). *Nitzschia* sp. mempunyai kandungan lipid tertinggi, manakala *C. cuspidata* mempunyai kandungan lipid terendah. Asid lemak juga menunjukkan perbezaan yang signifikan ($p < 0.05$) dalam Omega-3 (PUFAn-3) di mana, diatom air laut mempunyai hasil Omega-3 yang lebih tinggi daripada diatom air tawar. Selain itu, jumlah protein dan karbohidrat adalah sama ($p > 0.05$) dalam diatom, kecuali

Nitzschia sp. dan *C. cuspidata* yang menunjukkan perbezaan yang signifikan (p <0.05) dalam jumlah protein dan karbohidrat.

Secara keseluruhan, kadar pertumbuhan, biojisim, kandungan lipid keseluruhan dan kualiti profil asid lemak, *Nitzschia* sp. (diatom air laut) adalah lebih baik daripada lima spesies yang lain. Hal ini menjadikannya sumber yang baik untuk lipid (28% DW), protein (32% DW) dan omega-3 (21.77 mg/g DW) dan ianya juga boleh digunakan sebagai bahan makanan dalam sektor akuakultur dan sebagai sumber bahan bakar bio.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

EPA	Eicosapentaenoic acid
SEM	Scanning electron microscopy
AA	Arachidonic acid
ALA	Alpha linolenic acid
UFA	Unsaturated fatty acid
SFA	Saturated fatty acid
SD	Standard deviation
TAG	Triacylglycerol
PUFA	Polyunsaturated fatty acid
MUFA	Monounsaturated fatty acid
FA	Fatty acid
TIFA	Total identified fatty acid
FAMEs	Fatty acids methyl esters
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
DAG	Diacylglycerol
DW	Dry weight
LM	Light microscope
FESEM	Field emission scanning electron microscopy
Min	Minute
S	Second
h	Hour

OD	Optical density
GCMS	Gas chromatography mass spectrophotometer
SGR	Specific growth rate
FW	Freshwater
MW	Marine water
et al.,	et alia / and others

LIST OF SYMBOLS

%	Percent
‰	Part per thousand
mL	Millilitre
L	Litter
G	Gram
°C	Degree Celsius
g/L	Gram per litter
mg/L	Milligram per litter
µg/L	Microgram per litter
µl	Microliter
µm	Micrometre
µmol	Micromole

CHAPTER 1

INTRODUCTION

Diatoms are photosynthetic organisms that can produce lipids in large amounts and within short periods of time, and these can be processed into both biofuels and other useful commercial products. Due to this reason, diatoms are considered as a potential source of renewable energy. One of the most important decisions to obtaining oil from microalgae is the choice of species.

To evaluate a diatom species for its prospective use in aquaculture and biodiesel production, two important characteristics need to be considered: firstly, growth rate, and secondly, biochemical composition. Microalgae including diatoms varied in their contents of lipid, depending on the type of strain and its relevant culture conditions (Kang et al., 2011). The different culture conditions and techniques resulted in broad biochemical diversity among diatom species. Lipid content increases considerably (doubles) when the cells are subjected to unfavourable culture conditions, such as light intensity, salinity, temperature, as well as nutrient starvation and carbon dioxide (Chiu et al., 2009; Widjaja et al., 2009 and Qin, 2005). Mangas-Sánchez and Adlercreutz, (2015) found the average lipid content of diatoms under normal conditions was 22.7% - 25% of dry weight while the average lipid content achieved was 44.6% of dry weight under stress conditions (Hu et al., 2008).

In spite of their abundance and diversity of diatoms (100,000 species), only few number of species have been examined for their lipid, protein and carbohydrate contents. In addition, most of studies focused on biomass production, lipid and fatty acid in individual species of microalgae under the different culture conditions, only a few studies have been performed under similar culture conditions. In current study, we have investigated the growth rate, biomass, lipid, protein, carbohydrate and fatty acid of some Malaysian marine and freshwater diatoms cultured under similar conditions. Data on some of the species examined in this study have not been reported previously, although data on some well-studied species are included for comparison.

1.1 Hypothesis

Null hypothesis: There is no difference in lipids, proteins, carbohydrates and fatty acids of marine and freshwater diatoms cultured under similar conditions.

Alternative hypothesis: There is difference in lipids, proteins, carbohydrates and fatty acids of marine and freshwater diatoms cultured under similar con

1.2 Research objectives

The main aim of this work is to investigate the growth rate, biomass, lipid, protein, carbohydrate and fatty acid in some of Malaysian marine and freshwater diatoms cultured under similar conditions as well as same experimental protocol for six species of marine and freshwater diatom. Thus the specific objectives are listed as below.

1. To isolate and identify some Malaysian marine and freshwater diatoms.
2. To determine growth rate and biomass of some marine and freshwater diatoms cultured under similar conditions.
3. To determine the total lipid, protein, carbohydrate and fatty acid of some marine and freshwater diatoms cultured under similar conditions.

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