THE FATTY ACID CONCENTRATION AND COMPOSITION ON THE GROWTH OF THREE CULTURED MICROALGAE SPECIES

NUR SAFAWATI ABD MANAB



TY OF MARITIME STUDIES AND MARINE SCIENCE UNIVERSITI MALAYSIA TERENGGANU 2013

1100091344

Pusat Pembelajaran Digital Sultanah Nur Zahirah (UMT Universiti Malaysia Terengganu.





1100091344

The fatty acid concentration and composition on the growth of three cultured microalgae species / Nur Safawati Abd Manab.

PUSAT PEMBELAJARAN DIGITAL SULTANAH NUR ZAHIRAH UNIVERSITI MALAYSIA TERENGGANU (UMT) 21030 KUALA TERENGGANU

 110009	1342
54	
	=
00	
 - A-	Lihat Sebelah

HAK MILIK Pusat pembelajaran dicital sultanah nur zahirah

THE FATTY ACID CONCENTRATION AND COMPOSITION ON THE GROWTH OF THREE CULTURED MICROALGAE SPECIES.

By

NUR SAFAWATI ABD MANAB

Research Report submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (Marine Biology)

Department of Marine Science Faculty of Maritime Studies and Marine Science UNIVERSITI MALAYSIA TERENGGANU 2013

This project report should be cited as:

Safawati, N.A.M. (2013). The fatty acid concentration and composition on the growth of three cultured microalgae species. Undergraduate Thesis, Bachelor of Science (Marine Biology), Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu, Terengganu. 65 pp.

No part of this project report may be reproduced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.

12

1100091344



DEPARTMENT OF MARINE SCIENCE FACULTY OF MARITIME STUDIES AND MARINE SCIENCE UNIVERSITI MALAYSIA TERENGGANU

DECLARATION AND VERIFICATION REPORT

FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled:

THE FATTY ACID CONCENTRATION AND COMPOSITION ON THE GROWTH OF THREE CULTURED MICROALGAE SPECIES by .NUR SAFAWATI BINTI ABD MANAB., Matric No. .UK 22533... have been examined and all errors identified have been corrected. This report is submitted to the Department of Marine Science as partial fulfillment towards obtaining the Degree BACHELOR OF SCIENCE (MARINE BIOLOGY), Faculty of Maritime Studies and

Marine Science, University Malaysia Terengganu.

Verified by:

Principal Supervisor

Name: Official stamp: ROSWATI MD AMIN (ph.D) PENSYARAH JABATAN SAINS MARIN FAKULTI PENGAJIAN MARITIM & SAINS MARIN UNIVERSITI MALAYSIA TERENGGANU

Second Supervisor Name: Official stamp:

PROF. MADYA DR. ZAINUDIN BIN BACHOK Timbalan Pengarah Institut Oseanografi dan Sekitaran Universiti Malaysia Terengganu 21030 Kuala Terengganu, Terengganu

Date: 12/6/2017

13 JUN 2013 Date: ...

ACKNOWLEDGEMENTS

First of all, I would like to express my ultimate gratitude to Allah S.W.T. for good health and blessings in completing the research project including surviving from any obstruction and misfortune until I able to finish the research project completely.

Secondly, I would like to express gratitude to my supervisors; Dr. Roswati Mohd Amin and Assoc. Prof. Dr. Zainudin Bachok for all the helps, supports and guidance that had been given along the period in completing the final year research project. The invaluable advice and supervision including technical supports given by my supervisors were invaluable and precious as I learn lots of things along the research period.

I also would like to wish a grateful thank to Assoc. Prof. Dr. Siti Aishah Abdullah and Mrs. Maizah Mohd Abdullah including Final Year Project Coordinator Dr. Saifullah Arifin Jaaman, Dr. Antonina Abdullah, Mr. Yong Jaw Chuen and Dr. Lee Jen Nie on advice and support given throughout the period in facilitate the research project process.

Moreover, I would like to wish deeply thank to the Mrs. Noor Zuliana Mohd Nor, Mr. Abdul Manaf Ahmad, Ms. Nurul Hana Mohd Bakri, Ms. Siti Hajar Abdul Hamid, Ms. Nur Hazwani Zakaria, Mr. Fathurrahman Lananan, Ms. Mardiah Hayati Yahaya, Mr. Azahari Muda, Ms. Noor Azariyah Mohtar, Mr. Hassan Rashid Ali, Mr. Wan Mohd Redhuan Wan Azmi, Mr. Abdul Jalal Bilal and Mr. Sainol Aimi Saidin for assistance during progress of the research project on microalgae culture and fatty acid production including supplying invaluable information, guidance, opinion and advice. In addition, I also would like to express speechless thanks to all of the lab assistants in Biodiversity Laboratory and Oceanography Laboratory especially Mr. Che Mohd Zan Husin, Mr. Abdul Manaf Ahmad and Mr. Suliman Kasim for supplying all the equipment and chemicals needed throughout the research project. On the other hand, I also would like to express the thank to the science officers in Institute of Oceanography and Environment (INOS) and Institute of Tropical Aquaculture (AKUATROP) for the guidance and cooperation during preparation, preliminary study and experimental period in Marine Pollution and Chemical Oceanography Laboratory (INOS) and Live Feed Laboratory (AKUATROP) including their permission in utilizing the existing facilities provided at the laboratories.

I also would like to express appreciation towards FRGS Research Grant (MOHE), Faculty of Maritime Studies and Marine Science, and Institute of Oceanography and Environment, Universiti Malaysia Terengganu on granting fund to the project entitled 'The fatty acid concentration and composition on the growth of three cultured microalgae species'. Plus, I would like to express the thanks to all my friends who always give support and advice all through this project especially in managing data analysis. I am hoping more project regarding the marine ecosystem would be continued in the future to update and maintain the quality of research in Marine Science Department of Faculty of Maritime Studies and Marine Science.

Finally, I would like to give precious gratitude and thank to my beloved families who always give moral support and understanding including financial supports so that I able to complete my final project successfully.

ii

TABLE OF CONTENTS

Contents		Page
ACKNOW	LEDGEMENTS	i
TABLE O	F CONTENTS	iii
LIST OF 1	TABLES	v
LIST OF F	TIGURES	vi
LIST OF A	ABBREVIATIONS	ix
LIST OF F	ORMULAE	х
ABSTRAC	CT	xi
СНАРТЕВ	R 1: INTRODUCTION	
1.1	Background of the Study	1
1.2	Status and Importance of the Study	4
1.2	Objectives of the Study	5
СНАРТЕН	R 2: LITERATURE REVIEW	
2.1	Division of Fatty Acid	6
2.2	Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA)	7
2.3	Importance of Fatty Acids in Marine Organisms	9
2.4	Nutritional Value of Microalgae	11
CHAPTEI	R 3: METHODOLOGY	

3.1	Algae Culture	1	3
-----	---------------	---	---

3.2	Classification of the Microalgae Species	13
3.3	Preparation and Analysis of Microalgae Culture	15
3.4	Filtration of Microalgae Cultures and Dry Cell Weight	17
	Determination	
3.5	Extraction Analysis	18
3.6	Gas Chromatography Analysis	20
3.7	Data Analysis	22

CHAPTER 4: RESULTS

	4.1	Growth Rate of Three Cultured Microalgae Species	23
2	4.2	The Fatty Acid Concentration of Three Cultured Microalgae Species	26
2	4.3	The Dominant Fatty Acid Content of Three Microalgae Species	37
	4.4	The Fatty Acid Concentration of n-3 and n-6 Fatty Acids	44
CHAPT	ΓER	5: DISCUSSIONS	
:	5.1	Microalgae Growth Rate Analysis Across Phase	46
	5.2	Fatty Acid Composition of Microalgae	48
CHAP	ГER	6: CONCLUSION AND RECOMMENDATIONS	52
REFE	REN	CES	55
APPEN	NDIX	KES	62
CURR	ICU	LUM VITAE	65

Pusat Pembelajaran Digital Sultanah Nur Zahirah (UMD) Universiti Malaysia Terengganu.

LIST OF TABLES

Table

- 4.1 The colour, size, and specific growth rate of analysed microalgae 24 species.
- 4.2 The fatty acid concentration (pg cell⁻¹) of *Chlorella* sp. at difference 30 phases; early log (EL), log (L), late log (LL) and early stationary (ES). Abbreviations as in Table 4.2; SAFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; Σ: total or summation; tFA: total fatty acid. Values represent means ± standard deviation (S.D.) of two samples (n = 2).
- 4.3 The fatty acid concentration (pg cell⁻¹) of Dunaliella sp. at difference 33 phases; early log (EL), log (L), late log (LL) and early stationary (ES). Abbreviations as in Table 4.3; SAFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; Σ: total or summation; tFA: total fatty acid. Values represent means ± standard deviation (S.D.) of two samples (n = 2).
- 4.4 The fatty acid concentration (pg cell⁻¹) of *Rhodomonas* sp. at 36 difference phases; early log (EL), log (L), late log (LL) and early stationary (ES). Abbreviations as in Table 4.4; SAFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; Σ : total or summation; tFA: total fatty acid. Values represent means ± standard deviation (S.D.) of two samples (n = 2).

Page

LIST OF FIGURES

Figure

3.1 The taxonomical classification of three microalgae species; *Dunaliella* 14sp., *Chlorella* sp., and *Rhodomonas* sp.

Page

- 3.2 Phases of algal growth dynamics; 1: lag phase, 2: exponential or log 16 phase, 3: phase of declining relative growth, 4: stationary phase and 5: death phase.
- 3.3 The sequence of fatty acid extraction analysis to obtain FAME. 19
- 4.1 The cell concentration of three microalgae species (cells ml^{-1}); 24 *Chlorella* sp. *Dunaliella* sp. and *Rhodomonas* sp. (mean \pm S.D.).
- 4.2 Concentrations of total fatty acid (pg cell⁻¹) for all microalgae species 28 (*Chlorella* sp. *Dunaliella* sp. and *Rhodomonas* sp.) at different phases; early log (EL), log (L), late log (LL) and early stationary (ES). Values are the means \pm S.D.
- 4.3 The fatty acid concentration (pg cell⁻¹) according to phases; early log 37 (EL), log (L), late log (LL) and early stationary (ES) with dominant fatty acid of *Chlorella* sp. Note that the alphabet stand for fatty acid division; A: saturated fatty acid (SAFA), B: monounsaturated fatty acid (MUFA), and C: polyunsaturated fatty acid (PUFA). Abbreviations as in Figure 4.3; C16:0: palmitic acid; C17:0: heptadecanoic acid; C18:0: stearic acid; C14:1: myristoleic acid; C16:1: palmitoleic acid; C18:1 n9c: oleic acid; C18:2n6c: linoleic acid; C18:3n3: linolenic acid; C20:3n6: eicosatrienoic acid. Values are the means \pm S.D. Note that the right side of y-axis displays the total amount of fatty acid division and its standard deviation.

- 4.4 The fatty acid concentration (pg cell⁻¹) according to phases; early log 39 (EL), log (L), late log (LL) and early stationary (ES) with dominant fatty acid of *Dunaliella* sp. Note that the alphabet stand for fatty division; A: saturated fatty acid (SAFA), B: monounsaturated fatty acid (MUFA), and C: polyunsaturated fatty acid (PUFA). Abbreviations as in Figure 4.4; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C14:1: myristoleic acid; C16:1: palmitoleic acid; C18:1n9c: oleic acid; C18:2n6c: linoleic acid; C18:3n3: linolenic acid; C20:5n3: eicosapentaenoic acid. Values are the means ± S.D. Note that the right side of y-axis displays the total amount of fatty acid division and its standard deviation.
- 4.5 The fatty acid concentration (pg cell⁻¹) according to phases; early log 41 (EL), log (L), late log (LL) and early stationary (ES) with dominant fatty acid of *Rhodomonas* sp. Note that the alphabet stand for fatty division; A: saturated fatty acid (SAFA), B: monounsaturated fatty acid (MUFA), and C: polyunsaturated fatty acid (PUFA). Abbreviations as in Figure 4.5; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C20:0: arachidic acid; C14:1: myristoleic acid; C18:1n9c: oleic acid; C20:1: eicosenoic acid; C20:3n6: eicosatrienoic acid; C20:5n3: eicosapentaenoic acid. Values are the means ± S.D. Note that the right side of y-axis displays the total amount of fatty acid division and its standard deviation.
- 4.6 The fatty acid composition according to phases; early log (EL), log (L), 43late log (LL) and early stationary (ES) and fatty acid division; saturated

fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in each microalgae species (%). Note that the alphabet stand for; A: *Chlorella* sp., B: *Dunaliella* sp., and C: *Rhodomonas* sp.

4.7 The fatty acid concentration $(pg \text{ cell}^{-1})$ of n-3 and n-6 fatty acids 44 according to phases; early log (EL), log (L), late log (LL) and early stationary (ES) in three microalgae species (*Chlorella* sp., *Dunaliella* sp. and *Rhodomonas* sp.). Values are the means \pm S.D.

LIST OF ABBREVIATIONS

ARA	Arachidonic acid
BF ₃	Boron tri-fluoride
CH_2Cl_2	Dichloromethane
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FASW	Filtered autoclave sea water
g	gram
GC-FID	Gas chromatography – Flame Ionization Detector
HUFA	Highly unsaturated fatty acid
ind.	individual
mg	milligram
ml	millimetre
	mmmette
MUFA	Monounsaturated fatty acid
MUFA n-3	
	Monounsaturated fatty acid
n-3	Monounsaturated fatty acid Omega-3
n-3 n-6	Monounsaturated fatty acid Omega-3 Omega-6
n-3 n-6 PAH	Monounsaturated fatty acid Omega-3 Omega-6 Polynuclear aromatic hydrocarbon
n-3 n-6 PAH <i>p</i> g	Monounsaturated fatty acid Omega-3 Omega-6 Polynuclear aromatic hydrocarbon picogram
n-3 n-6 PAH <i>p</i> g PUFA	Monounsaturated fatty acid Omega-3 Omega-6 Polynuclear aromatic hydrocarbon picogram Polyunsaturated fatty acid

LIST OF FORMULAE

Concentration of fatty acid (C_{FA}) = $A_S/A_{IS} \times C_{IS}/W_S$

Where; A_S: peak area of fatty acid in the sample in chromatogram.

A_{IS}: peak area of internal standard in chromatogram.

C_{IS}: concentration of internal standard (mg)

W_S: weight of sample (g)

(eq. 1)

Cell density (cell/ml) =
$$C \times A_t$$

Where: C: total number of cells counted in the counting unit

 A_t : area of both grids used, mm² (18 mm²)

 A_s : area of counting unit used, mm² (0.25 mm x 0.25 mm)

S: number of counting units counted (24)

V: volume of sample under the grids used, ml (0.0018 ml) (eq. 2)

Growth rate (
$$\mu$$
)

$$= \ln X_2 - \ln X_1$$

$$t_2 - t_1$$
(eq. 3)
Division time (t_g)

$$= 0.6931$$

$$\mu$$
(eq. 4)

Where; X: number of cells

 μ : growth rate

t: time in days

ABSTRACT

Microalgae contain various types of essential fatty acid that functional in enhancing the growth and development of juvenile zooplankton. Three microalgae species had been culture with controlled condition and the culture method was inoculated with f/2medium. The growth of microalgae culture affects the composition and component of fatty acid content. Fatty acid content in the analysis differs between microalgae species and across the growth phases due to different in microalgae strains, culture conditions and relative distribution at major lipid classes. The highest specific growth rate was dominated by Dunaliella sp. followed by Rhodomonas sp. and Chlorella sp. with 0.41 ind.d⁻¹, 0.22 ind.d⁻¹ and 0.20 ind.d⁻¹ value respectively. On the other hand, the highest cell density dominated by Chlorella sp. followed by Rhodomonas sp. and Dunaliella sp. Among the three species studied, the highest fatty acid contents dominated by *Rhodomonas* sp. where it displayed the highest total fatty acid contents $(1075.578 \text{ pg cell}^{-1})$ in early log (EL) phase followed with Dunaliella sp. and Chlorella sp. In n-3 and n-6 fatty acids, Rhodomonas sp. also high in quantity across phases except in late log (LL) phase where the phases dominated by Chlorella sp. The fatty acids from polyunsaturated fatty acid (PUFA) division play main role in fatty acid contents as the highest contributor to fatty acid contents were from PUFA. The results obtained were significant in all microalgae species across four phases (p < 0.05).

Kepekatan dan Komposisi Asid Lemak pada Pertumbuhan Tiga Spesis Microalga yang Dikultur

ABSTRAK

Mikroalga mengandungi pelbagai jenis asid lemak yang bermanfaat dimana ia berfungsi dalam meningkatkan tumbesaran dan pertumbuhan zooplankton juvenil. Tiga spesies mikroalga telah dikultur dengan kondisi terkawal dan kaedah pengkulturan dinokulasi dengan f/2 media. Tumbesaran pengkulturan mikroalga memberi kesan kepada komposisi dan komponen kandungan asid lemak. Kandungan asid lemak di dalam analisis berbeza diantara mikroalga spesies dan seluruh fasa pertumbuhan disebabkan oleh perbezaan strain microalgae, kaedah pengkulturan dan pengedaran relatif di dalam kelas utama lipid. Kadar pertumbuhan tertentu didapati tertinggi di dalam spesies Dunaliella sp. diikuti dengan spesies Rhodomonas sp. dan Chlorella sp. masing-masing dengan nilai 0.41 ind.d⁻¹, 0.22 ind.d⁻¹ dan 0.20 ind.d⁻¹. Sebaliknya, ketumpatan sel tertinggi dikuasai oleh Chlorella sp. diikuti oleh Rhodomonus sp. dan Dunuliellu sp. Antara ketiga-tiga spesies yang dikaji, kandungan asid lemak vang tertinggi dikuasai oleh Rhodomonas sp. di mana ia memapaparkan jurnlah kandungan asid lemak tertinggi (1075.578 pg sel⁻¹) dalam fasa log awal (EL) diikuti dengan Dunaliella sp. dan Chlorella sp. Dalam n-3 dan n-6 asid lemak, Rhodomonas sp. juga didapati tertinggi dalam kuantiti di seluruh fasa kecuali dalam fasa log akhir (LL) mana fasa tersebut dikuasai oleh Chlorella sp. Asid lemak dari asid lemak politaktepu (PUFA) memainkan peranan utama dalam kandungan asid lemak dan bertindak sebagai penyumbang tertinggi kepada kandungan asid lemak. Keputusan yang diperolehi adalah signifikan dalam semua spesis mikroalga diantara empat fasa (p < 0.05).