UNIVERSITI PUTRA MALAYSIA

LIPID PRODUCTION IN MARINE MICROALGAE UNDER DIFFERENT SALINITY, TEMPERATURE AND NUTRIENT LEVELS

NURUL SALMA BINTI ADENAN

IB 2012 16

LIPID PRODUCTION IN MARINE MICROALGAE UNDER DIFFERENT SALINITY, TEMPERATURE AND NUTRIENT LEVELS

By

NURUL SALMA BINTI ADENAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

JULY 2012

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

LIPID PRODUCTION IN MARINE MICROALGAE UNDER DIFFERENT SALINITY, TEMPERATURE AND NUTRIENT LEVELS

By

NURUL SALMA BINTI ADENAN

July 2012

Chairman: Professor Fatimah Md. Yusoff, PhD

Institute: Bioscience

The marine microalgae *Chlorella* sp. (UPMC-A0013) and *Chaetoceros calcitrans* (UPMC-A0010) contain relatively high lipid levels (15.0 to 20.0% of dry weight) that can contribute as important components for the formulation of feed in aquaculture industry. However, lipid levels in microalgae vary according to the culture conditions. This study was carried out to determine the various environmental factors that control the growth and lipid contents of marine *Chlorella* sp. and *C. calcitrans* by subjecting the algae to different levels of stress in terms of salinity and temperature changes and nutrient depletion in their early stationary phase.

The effects of salinity stress on lipid production of *Chlorella* sp. and *C. calcitrans* were performed by culturing both microalgae species in Conway medium of various salinity levels (15, 20, 25, 30, 35 and 40‰). The cultures were centrifuged and resuspended into culture medium of lower salinity levels of -5‰ ($S1_i$) and -10‰

($S2_i$) from the initial salinity levels in their early stationary phase. The highest growth rate ($P<0.05$) of *Chlorella* sp. and *C. calcitrans* were observed at 25‰ ($\mu=0.37$ day^{-1}) and 30‰ ($\mu=0.28$ day^{-1}), respectively. Salinity changes from S_{30} to $S1_{30}$ and $S2_{30}$ increased $P<0.05$) the production of total lipid in *Chlorella* sp. to 10.0% and 19.0% of d.w., respectively. Total lipid of *C. calcitrans* increased significantly ($P<0.05$) to 13.2% of d.w. when stressed to $S2_{30.}$

Prior to the temperature stress, marine *Chlorella* sp. and *C. calcitrans* were cultivated at 20, 25, 30 and 35^oC (T_i). Then, the microalgae were shifted to higher temperature of +5^oC (TS_i) from the initial temperature levels in their early stationary phase. Before stress, *Chlorella* sp. showed the highest growth rate ($\mu= 0.35$ day^{-1}, $P<0.05$) at 25^oC (T_{25}). Total lipid of *Chlorella* sp. significantly increase ($P<0.05$) from 31.0% of d.w. (T_{25}) to 41.0% of d.w (TS_{25}). Diatom, *C. calcitrans* showed the highest growth rate at T_{30} ($\mu= 0.26$ day^{-1}) and lipid content increased significantly ($P<0.05$) from 31.0% to 39.0% after stress (TS_{30}).

The effects of nutrient stress on lipid production was determined by culturing the algae into Nitrogen and Phosphorus deprived Conway medium (25.0% reduction = N_{25} and P_{25}; 50.0% reduction = N_{50} and P_{50}; 75.0% reduction = N_{75} and P_{75}) in their early stationary phase. Lipid concentrations significantly increased ($P<0.05$) by 9.0% in both cultures subjected to 75% nitrogen and phosphorus reduction (N_{75} and P_{75}). This study illustrated that various growth conditions such as salinity and temperature changes, and reduction in nutrient concentration enhanced lipid content of *Chlorella* sp. and *C. calcitrans*.

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

PENGHASILAN LIPID OLEH MIKROALGA MARIN DI BAWAH TAHAP KEMASINAN, SUHU DAN NUTRIEN BERBEZA

Oleh

NURUL SALMA BINTI ADENAN

Julai 2012

Pengerusi : Professor Fatimah Md. Yusoff, PhD

Institut : Biosains

marin mikroalga, *Chlorella* sp. (UPMC-AA0013) dan *C. calcitrans* (UPMC-AA0010) mengandungi kadar lemak yang tinggi (15-20% berat kering) yang boleh diaplikasikan sebagai komponen penting dalam pembuatan dan penghasilan makanan bagi industri akuakultur. Namun begitu, kadar lipid yang dihasilkan bergantung kepada kaedah pengkulturan. Dengan itu, penentuan pelbagai faktor pengkulturan yang boleh mengawal kadar pertumbuhan dan penghasilan lipid adalah penting. Kadar pertumbuhan dan penghasilan lipid oleh *Chlorella* sp. dan *C. calcitrans* dilakukan dengan mengaplikasikan beberapa tahap tekanan pada pembolehubah pertumbuhan, yang merangkumi perubahan tahap kemasinan dan suhu, serta pengurangan nutrisi di awal fasa pertumbuhan mendatar.

Kajian kesan perubahan tahap kemasinan terhadap pengeluaran lipid bagi *Chlorella* sp. dan *C. calcitrans* dengan mengkultur kedua-dua mikroalga di dalam media Conway yang mengandungi tahap kemasinan asal (S_i) yang berbeza iaitu 15, 20, 25, 30, 35 dan 40‰. Kultur diemparkan dan dilarutkan ke dalam media yang mempunyai tahap kemasinan yang rendah, -5‰ ($S1_i$) dan -10‰ ($S2_i$) dari kepekatan asal. *Chlorella* sp. dan *C. calcitrans* menunjukkan kadar pertumbuhan yang tinggi pada 25‰ (μ = 0.37 $hari^{-1}$) dan 30‰ (μ=0.28 $hari^{-1}$). *Chlorella* sp. menunjukkan peningkatan pengeluaran lipid kepada 10.0% and 19.0% berat kering setelah berlaku perubahan tahap kemasinan dari S_{30} to $S1_{30}$ dan $S2_{30}$. Jumlah pengeluaran lipid bagi *C. calcitrans* meningkat kepada 13.2% berat kering setelah tahap kemasinan diubah ke $S2_{30}$.

Chlorella sp. dan *C. calcitrans* dikultur pada suhu 20, 25, 30 and 35^oC. Kemudian, peningkatan suhu sebanyak +5^oC (TS_i) dari suhu asal (T_i) dilakukan pada peringkat awal fasa pertumbuhan mendatar. Kadar pertumbuhan *Chlorella* sp. adalah μ= 0.35 day^{-1} ($P<0.05$) pada suhu 25^oC sebelum suhu dinaikkan. Kadar lipid meningkat dari 31.0% berat kering pada TS_{25} ke 41.0% berat kering selepas suhu dinaikkan ke 30^oC (TS_{25}). *Chaetoceros calcitrans* menunjukkan kadar pertumbuhan yang tinggi pada suhu T_{30} (μ= 0.26 $hari^{-1}$). Selepas suhu dinaikkan, kadar lipid meningkat dari 31.0% ke 39.0% berat kering pada TS_{30}.

Dalam menentukan kesan pengurangan nutrisi terhadap kadar penghasilan lemak, *Chlorella* sp. marin dan *C. calcitrans* dikultur ke dalam media Conway dan dilarutkan ke dalam kultur media yang mengandungi beberapa siri kepekatan

nitrogen dan fosforus yang dikurangkan (pengurangan 25.0% = N_{25} dan P_{25}; pengurangan 50.0% = N_{50} dan P_{50}; pengurangan 75.0% = N_{75} dan P_{75}) pada awal fasa pertumbuhan mendatar. Peratusan lipid bagi kedua-dua spesis didapati meningkat sebanyak 9.0% berat kering apabila kepekatan nitrogen dan fosforus dikurangkan sehingga 75.0%. Kajian ini menunjukkan perubahan tahap kemasinan dan suhu serta pengurangan kadar nutrisi dapat meningkatkan penghasilan peratusan lipid yang tinggi dalam *Chlorella* sp. marin dan *C. calcitrans*.

ACKNOWLEDGEMENTS

Assalamualaikum**,**

First of all thank you Allah, for your love and guidance I have successfully completed my laboratory work and finally, this thesis.

My sincere gratitude goes to my supervisors, Prof. Dr Fatimah Md. Yusoff and Prof. Dato' Dr Mohamed Shariff Mohamed Din for your guidance and encouragement throughout the research. Special thanks also extended to all my colleagues for their assistance, encouragement and friendship, I love you guys. To lecturers, researchers and science officers who were involved directly or indirectly throughout research, thank you for you help and advice.

Last but not least, I would like to acknowledge with thank and love to my family and husband for all their love, support and the tremendous efforts they have put to materialize this thesis.

I certify that an Examination Committee has met on 26th July 2012 to conduct the final examination of Nurul Salma Binti Adenan on her Master in Science thesis entitled "Lipid Production In Marine Microalgae Under Different Salinity, Temperature And Nutrient Levels" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980. The committee recommends that the student be awarded the Master of Science.

Members of the Examination committee were as follows:

Aziz Arshad, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mohamad Pauzi Zakaria, PhD
Professor
Faculty of Environmental Science
Universiti Putra Malaysia
(Internal examiner)

Che Roos Saad, PhD
Associate Professor
Faculty Agriculture
Universiti Putra Malaysia
(Internal examiner)

Wan Maznah Wan Omar, PhD
Associate Professor
School of Biological Sciences
Universiti Sains Malaysia
Malaysia
(External examiner)

SEOW HENG FONG
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Fatimah binti Md. Yusoff, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Mohamed Shariff bin Mohamed Din, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NURUL SALMA BINTI ADENAN

Date: 26 July 2012

TABLE OF CONTENTS