UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **LALITHA A/P MANIAM** (I.C/Passport No: **841109-10-5356**)

Registration/Matric No: **SGF090003**

Name of Degree: **MASTER OF BIOTECHNOLOGY**

Title of Project Paper/Research Report/Dissertation/Thesis ("**Growth and Biochemical Composition of Selected Algae in Palm Oil Mill Effluent**"):

Field of Study: **Algae Biotechnology**

I do solemnly and sincerely declare that:

1) I am the sole author/writer of this Work;
2) This Work is original;
3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date

Subscribed and solemnly declared before,

Witness's Signature Date

Name : PROF DR.PHANG SIEW MOI

Designation : PROFESSOR

ABSTRACT

The palm oil industry in Malaysia is one of the largest producers of agroindustrial wastewater known as Palm Oil Mill Effluent (POME). POME is highly organic in content which is acidic, thick, dark in colour, with high levels of chemical oxygen demand, ammonical nitrogen, orthophosphate, nitrate, nitrite, total suspended solids, and total solids. Due to high organic load, the raw POME has to be digested anaerobically to reduce the pollutant level, in order to meet the effluent discharge standard for palm oil mills. The objective of the present study is to investigate the potential of selected microalgae to grow in POME and anaerobic liquor (AL)as well as to assess the biochemical composition of the biomass and finally the percentage of pollution reduction obtained. Nine microalgae strains from the University of Malaya Algae Culture Collection (UMACC) which are *Chlorella vulgaris* UMACC 001, *Scenedesmus* UMACC 010, *Scenedesmus* UMACC 036, *Scenedesmus* UMACC 068, *Ankistrodesmus convolutus* UMACC 101, *Nannochloris bacillaris* UMACC 109, *Chlorococcum oviforme* UMACC 110, *Chlamydomonas augustae* UMACC 246 and *Chlorella* UMACC 300 were screened for growth and biochemical composition using flask cultures. Of nine strains, *Chlorella* UMACC 300 (specific growth rate (μ) $=0.35\pm0.02 d^{-1}$ and 39.88±2.73%DW of lipid), *Chlorella vulgaris* UMACC 001(μ=0.32±0.02 and 40.61±2.78%DW of lipid), *Scenedesmus* UMACC 036 ($\mu=0.30\pm0.05 d^{-1}$ and 32.75±1.01%DW of lipid) and *Ankistrodesmus convolutus* ($\mu=0.28\pm0.02 d^{-1}$ and 38.89 ±2.70%DW of lipid) were selected to grow in different concentrations of POME and anaerobic liquor. The *Chlorella* UMACC 300 showed better tolerance to grow in higher concentrations of POME and AL. *Chlorella* UMACC 300 which grew in 25% AL (Bold Basal Medium, BBM) produce biomass of 677.33±11.37 mg L^{-1} with 39.98±0.38 %DW protein, 19.12 ± 0.12 %DW carbohydrate and 43.44±0.92 %DW lipid on day 16 of culture period. This *Chlorella* UMACC 300

also produced higher pollution reduction when compared with other three selected microalgae, whereby it attained 87.66±0.59% reduction of chemical oxygen demand (COD), 65.38±2.51% ammonical nitrogen, 78.56±6.54% orthophosphate, 45.24±4.12 % nitrate and 42.13±11.81% nitrite. The second best strain which grew in different concentrations of POME and AL was *Chlorella vulgaris* UMACC 001 followed by *Scenedesmus* UMACC 036 and *Ankistrodesmus convolutus* respectively. The results obtained from this study shows that *Chlorella* UMACC 300 which was isolated from POME is tolerant of POME and AL and may be a potential species to be used for POME and AL treatment.

ABSTRAK

Industri kelapa sawit merupakan penyebab utama kepada bahan pencemar agroindustri yang dikenali sebagai efluen kilang kelapa sawit (POME). POME merupakan pencemar yang mempunyai kandungan organic yang tinggi dan bersifat asid, pekat, berwarna gelap dan mengandungi keupayaan kimia oksigen, ammonikal nitrogen, orthofosfat, nitrat, nitrit, total suspended solid and total solid. Disebabkan oleh kandungan organik yang tinggi, POME perlu dicerna secara anaerobik untuk mengurangkan kandungan bahan pencemar sebelum disalur keluar dari kilang pemprosesan minyak kelapa sawit, bagi memenuhi 'Discharge Standards' yang ditetapkan untuk kilang kelapa sawit. Objektif utama dalam kajian ini adalah untuk mengkaji potensi mikroalga terpilih untuk bertumbuh di media POME dan POME dicerna (AL) yang disediakan menggunakan kepekatan yang berbeza, bagi menilai kandungan biokimia and peratusan penurunan kandungan pencemar. Untuk itu, sembilan microalga dari 'University Malaya Algae Culture Collection' ia itu *Chlorella vulgaris* UMACC 001, *Scenedesmus* UMACC 010, *Scenedesmus* UMACC 036, *Scenedesmus* UMACC 068, *Ankistrodesmus convolutus* UMACC 101, *Nannochloris bacillaris* UMACC 109, *Chlorococcum oviforme* UMACC 110, *Chlamydomonas augustae* UMACC 246 and *Chlorella* UMACC 300 telah dikaji untuk mengenalpasti kandungan biokimia menggunakan sistem kultur. Daripada sembilan mikroalga *Chlorella* UMACC 300 (kadar pertumbuhan sesifik (μ)=0.35±0.02d^{-1} dan 39.88±2.73%DW lipid), *Chlorella vulgaris* UMACC 001(μ=0.32±0.02 dan 40.61±2.78%DW lipid), *Scenedesmus* UMACC 036 (μ=0.30±0.05d^{-1} dan 32.75±1.01%DW lipid) and *Ankistrodesmus convolutus* (μ=0.28±0.02d^{-1} dan 38.89±2.70%DW of lipid) telah dipilih untuk dikulturkan di dalam POME dan AL media yang disediakan menggunakan pelbagai kepekatan. *Chlorella* UMACC 300 menunjukkan toleransi yang tinggi untuk tumbuh di dalam POME dan AL yang berkepekatan tinggi. *Chlorella* UMACC 300 turut menunjukkan

pertumbuhan yang tinggi di dalam 25% AL (BBM) dengan 448.00±18.33mg L biomass, 27.70±0.93%DW protein, 1.60±0.07%DW karbohidrat dan 30.67±1.38%DW lipid. Mikroalga ini turut mencatatkan peratusan penurunan kandungan pencemar yang tinggi, dimana 87.66±0.59% penurunan COD, 65.38±2.51% ammonikal nitrogen, 78.56±6.54% orthofosfat, 45.24±4.12% nitrat and 42.13±11.81% nitrit. Mikroalga *Chlorella vulgaris* UMACC 001 menujukkan toleransi kedua tetinggi untuk bertumbuh di dalam POME dan di dalam AL. *Chlorella* UMACC 300 merupakan mikroalga yang mempunyai toleransi yang tinggi untuk bertumbuh di dalam POME media dan dapat mencapai peratusan penurunan pencemar yang tinggi. Keputusan yang diperolehi melalui kajian ini menunjukkan bahawa mikroalga yang berasal daripada POME mempunyai toleransi yang tinggi untuk bertumbuh di dalam POME dan di dalam AL, dimana mikroalga ini lebih sesuai untuk digunakan merawat POME dan AL.

ACKNOWLEDGEMENTS

First and foremost, I take great pleasure and honour to express my sincere appreciation to my main supervisor Professor Dr. Phang Siew Moi for giving me this opportunity to work on this project under Malaysian Palm Oil Board (MPOB). Prof. Dr. Phang Siew Moi as my supervisor, providing me a motivation, enthusiastic, continuous support, encouragement and also a proper guidance, ideas and comments throughout this experiment period. I am also would like to thank her for permitting me to use the equipments and facilities available in Algae Research Laboratory during the entire period of my bench work. I am also heartily thankful to my co-supervisors, Professor Dr. Chu Wan Loy from International Medical University (IMU) for his guidelines and sharing his valuable experiences in the field of microalgae research and also special thanks to Dr. Loh Soh Khaeng from Malaysian Palm Oil Board (MPOB) for her support to complete this project.

Besides that I would also like to thank MPOB for funding this experiment by supplying the chemicals and other important utilities needed throughout the experiment period, and also for their help in providing the transportation to me for the POME sample collection. Special thanks to Miss.Azreena Idris, Research Officer (RO) from MPOB for her support and guidelines throughout this experiment. Not forgetful the Tennamaram Estate Palm Oil organization which permitting me to collect the raw POME sample and digested POME sample for this experiment and also to the worker who help me during the sample collection.

I also would like to thank all the members of Algae Research Laboratory and Institute of Oceans and Earth Sciences (IOES) or their guidelines and sharing knowledge throughout this study. I would like to make a special reference to my dearest friend Saraswathy Ramachandaran, Teoh Ming Li, Pei San and Vijeysri for their

assistance especially when I am dealing with chemicals and equipment at Algae Research Laboratory.

Last but not least, I also would like to express my deepest gratitude and love to my father Mr. Maniam , my mother Madam. Valliammah, my brother Mr. Vishnu and also to my beloved husband Mr. Suresh and my daughter Lavannya for their unconditional love, patience, understanding, and sacrifices and support to me throughout this study. Thanks again to all of them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATONS

BBM	-	Bold' Basal Medium
CDM	-	Clean Development Mechanism
CER	-	Certified Emission Reduction
Chl-a	-	Chlorophyll-a
COD	-	Chemical Oxygen Demand
CPO	-	Crude Palm Oil
DEE	-	Department of Environmental Enforcement
DW	-	Dry Weight
EQA	-	Environmental Quality Act
FA	-	Fatty Acid
FAME	-	Fatty Acid Methyl Esters
FFB	-	Fresh Fruit Bunch
FGB	-	First Generation Biofuel
GC	-	Gas Chromatography
GHG	-	Green House Gases
GW	-	Global Warming
HEPES	-	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HRAP	-	High Rate Algal Pond
HRT	-	Hydraulic Retention Time
IPCC	-	Intergovernmental Panel for Climate Changes
MAS	-	Membrane Anaerobic System
MUFA	-	Monounsaturated Fatty Acid

NaOH - Sodium hydroxide

OD - Optical Density

pH - Potential of hydrogen

PHA - Polyhydroxyalkanoates

POME - Palm Oil Mill Effluent

PUFA - Polyunsaturated Fatty Acid

RPM - Rotates per Minute

SBR - Sequencing Batch Reactor

SFA - Saturated Fatty Acid

SGB - Second Generation Biofuel

STR - Solid Retention Time

TDS - Total Dissolved Solid

TGB - Third Generation Biofuel

TS - Total Solid

TSS - Total Suspended Solid

TVS - Total Volatile Solid

UMACC -University of Malaya Algae Culture Collection

LIST OF SYMBOL AND UNITS

%	-	percent
µ	-	specific growth rate
µg	-	micro gram
cell / mL	-	cells per millimetre
d^{-1}	-	per day
g	-	gram
mg / L	-	milligram per liter
^{o}C	-	degree Celcius
OD_{620nm}	-	Optical density at 620nm
psi	-	pound per square inch
Rpm	-	revolution per minute
v/v	-	volume per volume
w/v	-	weight per volume

LIST OF APPENDICES