STUDY ON GROWTH RATE OF *Spirulina platensis* CULTURE USING WASTEWATER FROM UNIVERSITI MALAYSIA SABAH (UMS) HATCHERY

NUR HAFIZAH BINTI RAMLY

Project thesis submitted in fulfillment of the requirements for the degree of Bachelor of Science with Honours (Aquaculture)

SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

2007

BORANG PENGESAHAN STATUS TESIS@

JUDUL: STUDY ON GROWTH RATE OF *Spirulina platensis* culture using wastewater from universiti malaysia sabah (UMS) hatchery.

Ijazah: Sarjana muda sains dengan kepujian (Akuakultur)

SESI PENGAJIAN: 2004/2007

Saya NUR HAFIZAH BINTI RAMLY
(HURUF BESAR)

mengaku membenarkan tesis (LPS/Sarjana/Doktor Falsafah)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[✓]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[ ]	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)	(TANDATANGAN PUSTAKAWAN)
Alamat Tetap: 888 VILLA CAHAYA, ANJUNG BIDARA, 78300 MASJID TANAH, MELAKA.	EN KENNEDY AARON AGUOL Nama Penyelia
Tarikh: 8 MEI 2007	Tarikh: 8 MEI 2007.

CATATAN: * Potong yang tidak berkenaan.
** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION FORM

I declare that the thesis is based on my original works and ideas except for the quotations and phrases, which have been acknowledged and the thesis has not been previously submitted for any other degree at Universiti Malaysia Sabah (UMS) or other institutions.

NUR HAFIZAH BT. RAMLY
HS 2004 – 1875
30 April 2007

EXAMINER DECLARATION

DECLARED BY

Signature

1. **SUPERVISOR**

   (Mr. Kennedy Aaron Aguol)

2. **EXAMINER 1**

   (Dr. Sitti Raehanah Mohd. Shaleh)

3. **EXAMINER 2**

   (Dr. Sujjat Al-Azad)

4. **DEAN**

   (Supt/KS. Assoc. Prof. Dr. Shariff A. Kadir S. Omang)

ABSTRACT

A study was undertaken to determine the suitability of using UMS Hatchery wastewater as an alternative medium for culturing *Spirulina platensis*, a blue green algae. The experiment was held indoor, under laboratory condition. The *Spirulina* cells were cultured using three types of treatments which were 100% Kosaric medium, 100% wastewater medium and mixed medium (50% v/v wastewater + 50% v/v Kosaric medium). The growth performance was studied during this experiment. The wastewater was analyzed to identify the contents of total nitrogen (mgL^{-1}) and phosphate (mgL^{-1}). The production cost for each treatments were calculated and also compared. Cell dry weight was best in wastewater at 0.2875±0.0343 gL^{-1} but there were no significant different ($p>0.05$) between 3 culture medium. Total nitrogen concentration (mgL^{-1}) also has no significant different but the highest nitrogen concentration was obtained from the Kosaric media at 5.1317±0.0288 mgL^{-1} before inoculation and 4.4870±0.0337 mgL^{-1} at the end of culture period. Phosphate concentration was highest in mixed medium (wastewater + Kosaric media) at 12.7883±0.3863 mgL^{-1} before inoculation and 8.9613±0.1788 mgL^{-1} at the end of culture period. Wastewater was the cheapest medium to produce at RM3.00 per 1L production and Kosaric was the most expensive medium to produce at RM7.73 per 1L production.

ABSTRAK

KAJIAN MENGENAI KADAR PERTUMBUHAN Spirulina platensis YANG DIKULTUR MENGGUNAKAN AIR BUANGAN DARI HATCERI UMS.

Satu kajian telah dijalankan untuk menentukan kesesuaian penggunaan air buangan Hatceri UMS sebagai media alternatif untuk mengkultur alga hijau biru, Spirulina platensis. Eksperimen dijalankan di dalam persekitaran makmal. Sel Spirulina platensis dikultur menggunakan tiga jenis media iaitu 100% media Kosaric, 100% media air buangan dan media campuran (50% media air buangan+ 50% media Kosaric). Prestasi pertumbuhan dikaji dalam eksperimen ini. Air buangan dianalisis untuk mengetahui kandungan jumlah nitrogen (TN) dan jumlah fosforus (TP). Kos penghasilan untuk setiap jenis media dikira dan dibandingkan. Pertumbuhan sel terbaik adalah media air buangan, 0.2875±0.0343 gL^{-1} tetapi tiada perbezaan yang signifikan antara ketiga-tiga media kultur tersebut. Kepekatan nitrogen, tidak mempunyai perbezaan yang signifikan di dalam semua media kultur akan tetapi kepekatan paling tinggi dicatat dari media Kosaric pada kepekatan nitrogen 5.1317±0.0288 mgL^{-1} di awal jangka masa pengkulturan dan 4.4870±0.0337 mgL^{-1} di akhir pengkulturan. Kepekatan fosforus pula adala paling tinggi di dalam media campuran (air buangan+ media Kosaric) dengan kepekatan di awal pengkulturan adalah 12.7883±0.3863 mgL^{-1} dan di akhir pengkulturan adalah 8.9613±0.1788 mgL^{-1}. Media air buangan adalah media yang paling murah kos pembuatannya iaitu RM3.00 untuk 1L media dan media Kosaric adalah yang paling mahal kos pembuatannya iaitu RM7.73 untuk 1L media.

ACKNOWLEDGEMENTS

Alhamdulillah, thanks to Allah, with His blessing, I managed to complete this final year project thesis on time.

Firstly, I would like to thank my supervisor, Mr. Kennedy Aaron Aguol for his guidance and advices in helping me throughout this project. To all lecturers who kindly lend me a hand, I greatly appreciate their helps especially Assoc. Prof. Dr. Shigeharu Senoo and Dr Yukinori Mukai. And also not to forget my examiners, Dr. Sitti Raehanah Mohd. Shaleh and Dr. Sujjat Al-Azad.

Besides that, lots of thanks to all BMRI staffs especially Mr. Asri, Mr. Ismail Tajul and also Mr. Junaidi who assisted me during laboratory works. I also want to thank all UMS Hatchery staffs and RAs for their helps and useful advices whenever I needed.

Very special thanks for my beloved fiancé, Mr. Mohd Syaffiq Sapiee, my parents, Mr. Ramly Ahmad and Mdm. Fatimah Ngah and also my family for their loves and supports.

Last but not least, to all my friends, especially Mr. Uganu Valentino Junior, Miss Nur Faiz and Miss Norhayati, I really appreciate your helps and moral supports.

LIST OF TABLES

	Page
2.1 Classes, genera and species of major currently named microalgae grown for food in fish aquaculture and their main utilization.	9
3.1 Details about the tilapia's wastewater taken for this experiment.	21
3.2 Random experimental layout.	27
3.3 Labeling of replicates.	28
4.1a Cost for each ingredient used to make Kosaric media.	46
4.1b Cost for A_5 micronutrients (was added in Kosaric ingredients calculation).	46
4.2 Cost for each ingredient added in wastewater as extra supplement.	47
4.3 Production cost comparison for three treatments.	48

LIST OF PHOTOS

Page

1.1 Filamentous *Spirulina platensis* under light microscope. 4

2.1 *Spirulina* product for aquaculture: Brine shrimp tablet fed with *Spirulina* as additional feed supplement. 8

2.2 Sodium bicarbonate could be replaced with bicarbonate of soda, that is available in the market. 19

3.1 Tilapia culture tank. 22

3.2 Water pump used to pump tilapias' wastewater to the filter. 22

3.3 *Spirulina platensis* during sub-culture period. 24

3.4 Random arrangement of bottles in laboratory. 28

3.5 Whatman filter and low pressure pump used to filter cells. 31

3.6 *Spirulina platensis* cells on membrane filter during drying in oven. 31

3.7 Samples in test tubes after added with mixed reagent during phosphate analysis. 35

3.8 Spectrophotometer used in total nitrogen and phosphate-phosphorus analysis. 35

LIST OF FIGURES

Page

3.1 Steps to prepare culture medium from tilapia's wastewater. 23

3.2 Starter culture cells transferring process. 24

3.3 Steps to inoculate *Spirulina platensis* cells in media for experimental purpose. 26

3.4 Transparent plastic bottle with aeration supply and pressure valve. 27

3.5 Method and formula to calculate dry weight sample. 30

3.6 Flow chart of total nitrogen analysis (Kitamura *et. al.*, 1982). 33

3.7 Flow chart of total phosphorus analysis (Parson *et al.*, 1984). 34

4.1 Growth performances of *Spirulina platensis* using three treatments over duration of 9 days. 39

4.2 Growth performance for *Spirulina platensis* cultured using Kosaric media over duration of 9 days. 40

4.3 Growth performances for *Spirulina platensis* cultured using wastewater media over duration of 9 days. 41

4.4 Growth performance for *Spirulina platensis* cultured using 50% wastewater media + 50% Kosaric media over duration of 9 days. 42

4.5 Nitrogen concentration in each media used to culture *Spirulina platensis*. 43

4.6 Phosphate concentration in each media used to culture *Spirulina platensis*. 44

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
>	More than
ANOVA	Analysis of Variance
AO	Aiba Ogawa medium
$CuSO_4$	Copper sulphate
GLA	Gamma linolenic acid
HCl	Hydrochloric acid
HROP	High-rate oxidation pond
$K_2S_2O_8$	Potassium peroxodisulfate
KH_2PO_4	Potassium dihydrogen phosphate
KNO_3	Potassium nitrate
LA	Linolenic acid
M	Molarity
mgL^{-1}	Milligram per liter
ml	Milliliter
N	Normal
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NED	N-1-naphthylenediamine hydrochloride
$NH_2C_6H_4SO_4NH_2$	Sulfanilamide
PBR	Photo bioreactor
PO_4-P	Phosphate-phosphorus
spp.	Species
RM	Ringgit Malaysia
RNA	Ribonucleic acid
SSM	Sea Saltpeter Medium
TN	Total Nitrogen
USD	U.S. Dollar
UMS	Universiti Malaysia Sabah
v/v	Volume per volume
w/w	Weight per weight
$ZnSO_4$	Zinc sulphate
β-carotene	Beta carotene
γ-linolenic acid	Gamma linolenic acid
$\mu mol\ m^{-1}s^{-1}$	Micro mol per meter per second

CONTENTS

	Page
DECLARATION	ii
EXAMINER DECLARATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
ABSTRAK	vi
CONTENTS	vii
LIST OF TABLES	xi
LIST OF PHOTOS	xii
LIST OF FIGURES	xiii
LIST OF SYMBOLS AND ABBREVIATIONS	xv
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	5
2.1 *Spirulina platensis*; nutritious microalgae	5
2.1.1 General Characteristics of Microalgae (*Spirulina platensis*)	5
2.1.2 The Importance of *Spirulina platensis*	6
2.1.3 The Growth and Life Cycle of *Spirulina platensis*	10
2.1.4 Environmental Factors That Affect the Growth of *S. platensis*	10
2.1.4a Physical Environment	10
2.1.4b Chemical Environment	11

2.1.5 Biochemical Composition of *Spirulina platensis* 12

2.1.6 Pigments in *Spirulina platensis* 13

2.1.7 Indoor Culture Management of *Spirulina platensis* 14

2.1.7a Culture Materials 14

2.1.7b Light 14

2.1.7c Temperature 15

2.1.7d Mixing 15

2.1.7e pH 16

2.1.7f Salinity 16

2.1.8 Control of Algal Development 16

2.1.8a Dry Weight Measurement 17

2.2 Cultivation of *Spirulina* on Wastewater 17

2.2.1 Constraint 17

2.2.2 Bicarbonate Needs 18

CHAPTER 3 METHODOLOGY 20

3.1 Pure Culture 20

3.2 Location 20

3.3 PreparationS 20

3.3.1 Medium Preparation 21

3.3.2 Sub-culture 23

3.4 Experimental Design 25

3.4.1 Culturing Process 25

3.4.2 Experimental Design Layout 27

3.4.3 Determining Cell Growth 29

3.5 Water Quality Determination 32

3.5.1 Total Nitrogen (TN) 32

3.5.2 Phosphate-Phosphorus (PO_4-P) 34

3.6 Cost Calculation 36

3.7 Statistical Analysis 37

CHAPTER 4 RESULTS 38

4.1 Growth Performance 38

4.1.1 Kosaric Media 39

4.1.2 Wastewater Media 40

4.1.3 Mixed Media (Wastewater + Kosaric Media) 41

4.2 Wastewater Samples' Analysis 42

4.2.1 Total Nitrogen Content (TN) 42

4.2.2 Phosphate-phosphorus Content 43

4.3 Cost Calculation Estimation and Comparison 44

4.3.1 Kosaric Media Production Cost 45

4.3.2 Wastewater Processing and Production Cost 46

4.3.3 Mixed Medium (Wastewater + Kosaric Media) Production Cost 47

4.3.4 Cost Comparison 47

CHAPTER 5 DISCUSSION 49

CHAPTER 6 CONCLUSION AND RECOMMENDATION 54

REFERENCES 55

APPENDIX 1 59

APPENDIX 2 60

APPENDIX 3 61

CHAPTER 1

INTRODUCTION

Richmond (2004) explained that any organisms with chlorophyll-*a* and a thallus not differentiated into roots, stem and leaves could be classified as an alga. Cyanobacteria are included in this definition, even though they are prokaryotic organisms. In applied phycology the term microalga refers to the microscopic algae and the oxygenic photosynthetic bacteria such as cyanobacteria, formerly known as Cyanophyceae.

Arthrospira (*Spirulina*), of which the most popular known species are *Arthrospira* (*Spirulina*) *platensis* and *Arthrospira* (*Spirulina*) *maxima*, are a group of cyanobacteria characterized by loosely spiral-shaped trichomes arranged in an open helix enclosed in a thin mucilaginous sheath (Castenholz, 1989). They can be found in a variety of environments, particularly in alkaline, brackish and saline waters. Of the few cyanobacteria that have the longest history of being utilized as part of the human diet, *Spirulina platensis* was the first cyanobacterium to be commercially cultivated using modern technology (Qiang Hu, 2004b). These fascinating characteristics were the main reason *Spirulina platensis* was proposed in this study which has high commercial value and ease of culture.

Spirulina platensis is widely known to contain nutritional biochemical compounds. After years of research, it was approved to be rich in protein and vitamin B_{12} and may be a nutritious addition to the often poor diet of people who live in developing countries such as Africa (Greenaway, 1990). It has relatively low percentage of nucleic acids which makes *Spirulina* a potential food items for persons suffering from coronary illness and obesity.

Spirulina pure culture or starter culture is cultivated using special made media with specific enrichment scheme. Some of the more common media based on chemical reagent widely used are Zarouk media, SSM (Sea saltpeter medium), Vonshak, Spirulina and AO (Ogawa and Aiba, 1977). The chemical reagents that are used in the media ingredients are expensive and it resulted in the high production cost (data from Fichersci.com). Therefore, efforts were made in this study to determine suitability of using hatchery wastewater as an alternative culture media. The outcomes of this study could promote lower production cost.

Wastewater contain high amount of nutrient. The major nutrients present in wastewater are phosphorus, ammonia, nitrite and nitrate. These nutrients are crucial for microalgae growth (Vonshak, 1997). In the past fifteen years, the use of microalgae and cyanobacteria for wastewater treatment has been reviewed by many researchers (Vonshak, 1997). These reviews demonstrate that algal cultures in high-rate oxidation ponds (HROP) for the removal of nitrogen and phosphorus in parallel with production of a useful algal biomass can be an interesting alternative to conventional tertiary wastewater treatment. The most abundant algae found in the water of naturally managed HROPs are generally of genera *Chlorella*, *Ankistrodesmus* and *Scenedesmus*. However, the removal of these small algae from the effluent can represent a major cost associated with algal cultivation on wastewater (Laliberté *et. al.*, 1997).

There are also other rational factors that contributed to the usage of wastewater in microalgae culture. Wastewater removal represents another important niche in which photoautotrophic microalgae are prominent. Using photosynthetic microalgae to take up the oxidized minerals released by bacterial action and, in turn, enrich the water with oxygen to promote an aerobic environment and reduce pathogens, makes good practical sense and could be well used in suitable locations the world over (Richmond, 2004).

The practice of using wastewater as culture media for microalgae would compliment efforts in reducing environmental pollutions to the surrounding area near the hatchery. It would also reduce cost in hatchery wastewater treatment.

The main objective of this study is to culture *Spirulina platensis* in different types of adjusted media using wastewater. The potential of replacing wastewater as *Spirulina* culture media may well be an alternative practice in the future to reduce its production cost.

Therefore, the objectives of this study are:

i. To identify the suitability of UMS hatchery waste water as an alternative culture medium for *Spirulina platensis*.

ii. To compare the production cost of *Spirulina platensis* culture using control medium and waste water medium.

Photo 1.1 Filamentous *Spirulina platensis* under light microscope (Source: Kennedy, 2006).

CHAPTER 2

LITERATURE REVIEW

2.1 *Spirulina platensis*, Nutritious Microalgae

2.1.1 General Characteristics of Microalgae (*Spirulina platensis*)

Spirulina platensis is the simplest plant living in water, soil and damp places (Greenaway, 1990). It may consist of one or a few cells with some made up of long line cells and form thin thread called filament. It contains chlorophyll and undergoes photosynthesis. It has its own colour produced by one or more pigments. This blue green alga is believed to be one of the first plants since the existence of earth.

Spirulina platensis belongs to the class Cyanophyceae; its cell structure and organization is typical of cyanobacteria (Qiang Hu, 2004b). The gram negative cell wall is composed of four layers with a major structural layer of peptidoglycan. Its central nucleoplasmic region contains carboxysomes, ribosome, and cylindrical bodies and lipids droplets. Thylakoid membranes associated with phycobilisomes are distributed peripherally in the cytoplasm. The peripheral region of the cell contains gas vacuoles and several other sub-cellular inclusions, such as polyphosphate granules and polyglucan granules.

Spirulina is categorized under the Genus Arthrospira, sub-section III, family Oscillatoriaceae and Order Oscillatoriales. It is microscopic in size, usually having a diameter between 1 to 12μm and length 3 to 20mm (Castenholz, 1989)

2.1.2 The Importance of *Spirulina platensis*

It is not known with accuracy when man began to use microalgae. The current use of these resources has three precedents: tradition, scientific and technological development, and the so-called, "green tendency" (Henrikson, 1998). In 1521, *Spirulina* was harvested from the Lake Texcoco, dried and sold as a flake cake for human consumption in a Tenochtitlán (today Mexico City) market (Ciferri, 1983). In 1940, the French phycologist P. Dangeard mentioned a cake called *dihé*, consumed by the people of the Kanembu tribe, near the African Lake Chad, in the sub-dessert area of Kanem (Ciferri, 1983). *Dihé* is a hardened cake of blue-green algae, collected at the banks of small ponds surrounding the lake and later on sun-dried.

Lately, the use of *Spirulina* has expended from its original application as human food supplements and animal feed to the production of fine chemicals for clinical diagnosis, biological research and cosmetic applications (Qiang Hu, 2004b). More recent studies of the therapeutic and health effects of *Spirulina* are expected to promote the application of this organism in the pharmaceutical and nutraceutical industries. It is anticipated that *Spirulina* will continue to act as the single most important species in the cyanobacterial biotechnology industry in the next decade or foreseenable future.

Even though *Spirulina* and other algal species have been used as food or food supplement for quite some time, its use as animal feed is more recent (Becker, 2004). Exception of cyanobacteria *Spirulina* as feed for ruminants such as cattle and sheep make it a better supplement. *Spirulina* also was used in studies on poultry farming. Significantly higher growth rates and lower non-specific mortality rate were observed in turkey poults fed with *Spirulina* diet. There were also studies reported of replacing soy protein with *Spirulina maxima* and *Spirulina platensis* to piglets weaned on a dry diet four to eight days of age to gain weight (Becker, 2004).

Some reports showed *Spirulina* and its enzymatic hydrolysates promote skin metabolism and prevent keratinization (Becker, 2004). *Spirulina* is a valuable source of linolenic acid, which can not be synthesized by animals or humans. This essential fatty acid has been connected with the stimulation of prostaglandin synthesis (Becker, 2004).

Spirulina is marketed and consumed in: Germany, Bangladesh, China, Myanmar, Vietnam, Brazil, Chile, Spain, France, Canada, Belgium, Egypt, United States, Ireland, Argentina, Philippines, India, Africa, and other countries, where public administration, sanitary organisms and associations have approved human consumption (Henrikson, 1998).

Photo 2.1 *Spirulina* product for aquaculture: Brine shrimp tablet fed with *Spirulina* as additional feed supplement (Source: hirakiUSA.com).