

**INFLUENCE OF NITROGEN SOURCES ON
GROWTH PROFILE AND THEIR KINETIC
STUDIES IN NEWLY ISOLATED ACIDOPHILIC
MICROALGAE, *COCCOMYXA DISPAR* AND
*SCENEDESMUS PARVUS***

LEE ZI XIN

UNIVERSITI SAINS MALAYSIA

JULY 2020



**PUSAT PENGAJIAN TEKNOLOGI
INDUSTRI UNIVERSITI SAINS
MALAYSIA**

**BORANG PENYERAHAN DISERTASI
MUTAKHIR SATU (1) NASKAH**

Nama penyelia: DR. MOHD ASYRAF KASSIM

Bahagian: TEKNOLOGI BIOPROSES

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh

Encik/Puan/Cik LEE ZI XIN

mengenai disertasinya sebagaimana yang dipersetujui oleh Panel Pemeriksa di Viva Vocanya.

2. Saya ingin mengesahkan bahawa saya berpuashati dengan pembetulan/pindaan yang dilaksanakan oleh calon.

Sekian, terima kasih.

(Tandatangan dan cop)

19/7/2020
(Tarikh)



**INFLUENCE OF NITROGEN SOURCES ON
GROWTH PROFILE AND THEIR KINETIC
STUDIES IN NEWLY ISOLATED ACIDOPHILIC
MICROALGAE, *COCCOMYXA DISPAR* AND
*SCENEDESMUS PARVUS***

by

LEE ZI XIN

A dissertation submitted in the partial fulfillment of the requirements for the degree of
Bachelor of Technology (B.Tech) in the field of Food Technology
School of Industrial Technology
Universiti Sains Malaysia

JULY 2020

DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

ZIXIN

LEE ZI XIN

JULY 2020

ACKNOWLEDGEMENT

The completion of this thesis could not have been possible without the participation and assistance of so many people whose names may not all be enumerated. First of all, I would like to take this opportunity to express my utmost gratitude to my supervisor, Dr. Mohd Asyraf Kassim who guided me throughout my research project in both practical and writing. Thousand thanks to his patience, support and encouragement throughout this year which are my biggest force to complete this thesis. I would also like to extend my sincere thanks to Tan Kean Meng and Ramizah Kamaludin who constantly guided me and gave me so many helpful tips to make my project runs smoothly.

Apart from that, my thanks also go to all staff in School of Industrial Technology especially Mr. Azmaizan bin Yaakub and Madam Najmah binti Hamid for their assistance by providing me all the necessary things that I need in the laboratory. I also appreciate all the lecturers who taught me throughout my four years of degree and provided me useful knowledge.

In addition, I would like to thank my family members who constantly giving me enough of care and support so that I can finish my project. Not to forget all my course-mates who supported me and lent their helping hands when I needed them. Last but not least, I would like to express my deepest thanks to all the rest that I did not mention for their willingness to help me during my project. Their contributions are sincerely appreciated and gratefully acknowledged.

ZIXIN

LEE ZI XIN

JULY 2020

TABLE OF CONTENTS

ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF SYMBOLS AND ABBREVIATIONS	x
ABSTRAK	xii
ABSTRACT	xiii
CHAPTER 1 INTRODUCTION	1
1.1 Research Background	1
1.2 Research Scope and Objectives	4
CHAPTER 2 LITERATURE REVIEW	5
2.1 Algae	5
2.2 Microalgae	6
2.3 Benefits of Microalgae	7
2.4 Microalgal Biochemical Composition	8
2.5 Factors Affecting Microalgae Cultivation	9
2.6 Nutrients	10
2.7 Nitrogen Source	12
2.7.1 Organic Nitrogen	14
2.7.1.1 Urea	15

2.7.2	Inorganic Nitrogen	16
2.7.2.1	Ammonium	16
2.7.2.2	Nitrate	18
2.8	Mathematical Modeling	20
CHAPTER 3 MATERIALS AND METHODS		23
3.1	Microalgae Culture and Cultivation Medium	23
3.1.1	Medium and Chemical Composition	23
3.1.2	Microalgae Culture	23
3.1.2.1	Morphology of Microalgae Strains	24
3.2	Preparation of Experimental Cultivation Medium	25
3.3	Cultivation of Microalgae in Different Nitrogen Sources	25
3.4	Analytical Method	26
3.4.1	Cell Biomass Calibration Curve	26
3.4.2	Growth Profile	28
3.4.3	Growth Kinetic Parameters	28
3.5	Kinetic Modeling	29
3.6	Statistical Analysis	30
CHAPTER 4 RESULTS AND DISCUSSION		31
4.1	Microalgae Morphology	31
4.2	Effect of Nitrogen Sources on Microalgae	32
4.3	Kinetic Modeling	41

CONCLUSION	48
4.4 Conclusion	48
4.5 Recommendation for Future Research	50
REFERENCES	52
APPENDICES	60

LIST OF TABLES

Table	Caption	Page
2.1	Biochemical composition of different species of microalgae.	8
2.2	Biomass yield achieved by using different forms of nitrogen sources.	14
4.1	The growth kinetic parameters of <i>Coccomyxa dispar</i> cultivated in Bold Basal Medium with different nitrogen sources.	33
4.2	The growth kinetic parameters of <i>Scenedesmus parvus</i> cultivated in Bold Basal Medium with different nitrogen sources.	35
4.3	Performance indices of the nonlinear model for <i>Coccomyxa dispar</i> and <i>Scenedesmus parvus</i> cultivated under 0.1% w/v sodium nitrate.	41
4.4	Predicted parameters of <i>Coccomyxa dispar</i> and <i>Scenedesmus parvus</i> cultivated in 0.1% w/v sodium nitrate of Bold Basal Medium (BBM).	42

LIST OF FIGURES

Figures	Caption	Page
2.1	Microscopic photographs (magnification 1:1000) of different algae species.	5
2.2	Macroalgae of Phaeophyceae found at Waisai Coast, Indonesia	6
2.3	Schematic representation of intracellular pathways involved in nitrogen uptake, storage, assimilation and partitioning in an algal cell.	13
4.1	<i>Coccomyxa dispar</i> under microscope with 40 x 10 resolution.	31
4.2	<i>Scenedesmus parvus</i> under microscope with 40 x 10 resolution.	31
4.3	Cell biomass (g L ⁻¹) of <i>Coccomyxa dispar</i> cultivated in Bold Basal Medium with different nitrogen sources.	33
4.4	Cell biomass (g L ⁻¹) of <i>Scenedesmus parvus</i> cultivated in Bold Basal Medium with different nitrogen sources.	35
4.5	Biomass productivity (g L ⁻¹ day ⁻¹) of <i>Coccomyxa dispar</i> and <i>Scenedesmus parvus</i> cultivated in Bold Basal Medium with different nitrogen sources.	40
4.6	Logistic model for <i>Coccomyxa dispar</i> cultivated in nitrogen-deficient medium supplemented with 0.1% of sodium nitrate.	43
4.7	Gompertz model for <i>Coccomyxa dispar</i> cultivated in nitrogen-deficient medium supplemented with 0.1% of sodium nitrate.	44
4.8	Baranyi-Robets model for <i>Coccomyxa dispar</i> cultivated in nitrogen-deficient medium supplemented with 0.1% of sodium nitrate.	44
4.9	Logistic model for <i>Scenedesmus parvus</i> cultivated in nitrogen-	46

	deficient medium supplemented with 0.1% of sodium nitrate.	
4.10	Gompertz model for <i>Scenedesmus parvus</i> cultivated in nitrogen-deficient medium supplemented with 0.1% of sodium nitrate.	47
4.11	Baranyi-Roberts model for <i>Scenedesmus parvus</i> cultivated in nitrogen-deficient medium supplemented with 0.1% of sodium nitrate.	47

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Caption
+	plus
-	minus
±	plus-minus
=	equal
%	percent
<	less than
°C	degree Celsius
e.g.	for example
H ₂ O	water
HCl	Hydrochloric acid
CO ₂	carbon dioxide
O ₂	oxygen
Mg ²⁺	magnesium ion
Ca ²⁺	calcium ion
K ⁺	potassium ion
(NH ₂) ₂ CO	urea
H ₂ CO ₃	carbonic acid
NH ₃	ammonia
NH ₄ ⁺	ammonium
NO ₃ ⁻	nitrate
NO ₂ ⁻	nitrite

Abbreviation	Caption
cm	centimetre
day ⁻¹	per day
DNA	deoxyribonucleic acid
EC	Enzyme commission number
g	gram
g L ⁻¹ day ⁻¹	gram per litre per day
h	hour
L min ⁻¹	litre per minute
Lux	luminous flux per unit area
mg L ⁻¹	milligram per litre
mL	millilitre
mM	millimolar
nm	nanometre
OD ₆₈₀	optical density at wavelength 680 nanometre
pKa	ion dissociation constant
R ²	correlation coefficient
RNA	ribonucleic acid
rpm	revolutions per minute
sp.	species
SD	standard deviation
USD	United States Dollar
μmol photons m ⁻² s ⁻¹	micro mol photons per metre square per second
v/v	volume per volume
w/v	weight per volume

**KESAN NITROGEN TERHADAP PROFIL PERTUMBUHAN DAN KAJIAN
KINETIK DUA MIKROALGA ASIDOFILIK, *COCCOMYXA DISPAR* DAN
*SCENEDESMUS PARVUS***

ABSTRAK

Biojisim mikroalga merupakan bahan mentah yang diperbaharui sesuai sebagai tenaga baharu pada masa depan. Walaubagaimanapun, penghasilan biojisim yang rendah tetap menjadi cabaran yang harus dikuasai dan beberapa faktor telah dikenalpasti untuk meningkatkan penghasilan biojisim. Nitrogen yang merupakan makronutrien menjadi unsur yang penting untuk dikaji kerana nitrogen yang sesuai dapat mengubah tapak jalan metabolik mikroalga dan seterusnya meningkatkan penghasilan biojisim. Walaupun banyak kajian tentang kesan nitrogen terhadap spesies mikroalga yang berbeza telah dijalankan, namun maklumat tentang kesan nitrogen terhadap spesies mikroalga *Coccomyxa dispar* dan *Scenedesmus parvus* masih kurang. Kajian ini telah dijalankan untuk mengkaji kesan nitrogen dengan mengkulturkan *Coccomyxa dispar* dan *Scenedesmus parvus* dalam Medium Bold Basal yang kekurangan nitrogen tetapi digantikan dengan amonium sulfate, sodium nitrat dan urea dalam kepekatan 0.1 peratus berat isipadu. Data kinetik pertumbuhan juga dibandingkan dengan menggunakan tiga model pertumbuhan (Logistik, Gompertz dan Baranyi-Roberts). Keputusan menunjuk bahawa kesan nitrogen terhadap penghasilan biojisim kedua-dua mikroalga adalah berbeza ketara. Sodium nitrat merupakan nitrogen yang terbaik untuk meningkatkan penghasilan biojisim bagi *Coccomyxa dispar* dan *Scenedesmus parvus* dengan menghasilkan produktiviti biojisim masing-masing sebanyak $0.080 \text{ g L}^{-1} \text{ hari}^{-1}$ and $0.037 \text{ g L}^{-1} \text{ hari}^{-1}$. Keputusan juga menunjukkan model Logistik paling sesuai kepada *Coccomyxa dispar* (R^2 0.9954) dan model Baranyi-Roberts paling sesuai kepada *Scenedesmus parvus* (R^2 0.9972) untuk meramalkan profil kadar pertumbuhan mikroalga.

INFLUENCE OF NITROGEN SOURCES ON GROWTH PROFILE AND THEIR KINETIC STUDIES IN NEWLY ISOLATED ACIDOPHILIC MICROALGAE, *COCCOMYXA DISPAR* AND *SCENEDESMUS PARVUS*

ABSTRACT

Microalgae are potential candidates to be sustainably developed in the future as a new energy resource. However, low biomass production remains a big challenge to be tackled. Several factors had been tested to increase biomass production including abiotic and biotic factors. Nitrogen is the macronutrient in which uptake is of particular importance. This is because by supplying suitable nitrogen sources, metabolic pathways can be manipulated thus expand the production capabilities of microalgae. Despite many studies conducted on effect of nitrogen sources in different microalgae species, there is still limited information on *Coccomyxa dispar* and *Scenedesmus parvus*. Thus, this research is conducted to determine the influence of nitrogen sources on two acidophilic microalgae strains, *Coccomyxa dispar* and *Scenedesmus parvus* through cultivating them in nitrogen-deficient Bold Basal Medium (BBM) supplemented with 0.1% w/v ammonium sulphate, sodium nitrate and urea. The growth data were fitted into three mathematical models (Logistic, Gompertz and Baranyi-Roberts) to analyse the cell growth profile. Results indicated that both microalgae strains were significantly influenced by the type of nitrogen sources. With the best nitrogen source (0.1% w/v sodium nitrate), the biomass productivity achieved by *C. dispar* ($0.080 \text{ g L}^{-1} \text{ day}^{-1}$) was almost double compared to *S. parvus* ($0.037 \text{ g L}^{-1} \text{ day}^{-1}$). Besides, Logistic model model was the most suitable kinetic growth model for *Coccomyxa dispar* with R^2 of 0.9954 and standard error of estimate of 0.0251 while Baranyi-Roberts model was the most suitable kinetic growth model for *Scenedesmus parvus* with R^2 of 0.9972 and standard error of estimate of 0.0098, to predict the growth profile when cultivated in 0.1% w/v sodium nitrate.