

**INFUENCE OF MASS TRANSFER TOWARDS
PILOT-SCALE SEMI-CONTINUOUS
CULTIVATION OF *Chlorella vulgaris***

KHOO CHOON GEK

UNIVERSITI SAINS MALAYSIA

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PILOT-SCALE SEMI-CONTINUOUS
CULTIVATION OF *Chlorella vulgaris***

by

KHOO CHOON GEK

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for the degree of
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On top of that, a good support system is important to surviving and staying sane in postgraduate study. I am fortunate to be accompanied and supported by many people, especially my fellow lab mates: Gaik Tin, Lee Muei, Janah, and Yong Yi for their timely suggestions, constructive criticism, and strong encouragement, as well as their friendship, which I appreciated enormously. In addition, awesome people from the nearby communities (Huey Tyng, Weng, Yeng Hok, Tow Leong, Yeek Chia, Mei

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Last and most importantly, I have an amazing family, unique in many ways, and the stereotype of a perfect family in many others, where the source of my life energy resides. My family deserves a lot of credits for always providing the kind of support the only family can give. Their understanding and immense support have been unconditional all these years, without whom this would not have been possible. To my parents (Mr. Khoo Yau Meng & Mdm. Chin Yeo Moi), thanks for the unconditional love and support. To my siblings (Kheeng Leeng, Choon Lian, Kheng Hock and Kheng Seng), thank you for all your patience, love and motivation. It has allowed me to overcome every obstacle I have encountered throughout this journey.

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TABLE OF CONTENTS

ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iv
LIST OF TABLES	xi
LIST OF FIGURES	xiv
LIST OF PLATE	xxii
LIST OF SYMBOLS	xxiii
LIST OF ABBREVIATIONS	xxvi
ABSTRAK	xxvii
ABSTRACT	xxx
CHAPTER 1 INTRODUCTION	1
1.1 Background of study	1
1.2 Problem statement	3
1.3 Research objectives	7
1.4 Scopes of study	8
1.5 Thesis organization	9
CHAPTER 2 LITERATURE REVIEWS	11
2.1 Microalgae	11
2.2 Factors to consider for microalgae strain selection	13
2.3 Conceptual study on microalgae growth in large-scale system	15
2.4 Microalgal culturing factors	18
2.4.1 Illumination	19

2.4.2	Culturing temperature	22
2.4.3	Nutrients	23
2.4.4	pH and salinity	24
2.4.5	Flow and mixing	26
2.4.6	Mass transfer	27
2.5	Cultivation strategy	28
2.5.1	Batch culture	29
2.5.2	Semi-continuous culture	30
2.5.3	Continuous culture	32
2.6	Large scale cultivation system design	33
2.6.1	Open pond system	33
2.6.2	Closed photobioreactor system	35
2.7	Challenges in microalgae scale-up cultivation	36
2.8	Macro-scale and micro-scale modelling of microalgae cultivation in tubular photobioreactor	37
2.8.1	Macro-scale modelling	38
2.8.2	Micro-scale modelling	41
	2.8.2(a) Basic kinetic equations: Primary models	41
	2.8.2(b) Dynamics kinetic equations: Secondary models	44
2.9	Potential industrial application of mathematic modelling	51
2.10	Microalgal biofuel production pathways	52
	2.10.1 Hydrothermal processing on microalgal biomass	54
	2.10.2 The properties of water in hydrothermal processing	58
2.11	Summary	60

CHAPTER 3	METHODOLOGY	61
3.1	Research design	61
3.2	Materials	63
3.2.1	Materials and chemicals	63
3.2.2	Equipment and instrumentation	65
3.3	Experimental setup	66
3.3.1	Bubble column photobioreactor	66
3.3.2	Hydrothermal carbonization reaction rig	68
3.4	Preliminary assessment of microalgae growth	69
3.4.1	Microalgae strain	70
3.4.2	Microalgae culture acclimatization	71
3.4.3	Selection of semi-continuous cultivation cycle	74
3.4.4	Influence of CO ₂ solubility towards cultivation pH	77
3.5	Microalgal cultivation in pilot-scale bubble column photobioreactor	82
3.5.1	Measurement of microalgae growth rate	84
3.5.2	Microscopy examination of microalgae cells	84
3.5.2(a)	Optical microscopy	84
3.5.2(b)	Transmission electron microscopy	85
3.5.3	Measurement of carbon dioxide biofixation rate	87
3.5.4	Measurement of carbon dioxide concentration	87
3.5.5	Measurement of nutrients concentration	88
3.6	Investigation of the influences of hydrodynamic and mass transfer phenomenon in pilot-scale microalgae cultivation	88

3.6.1	Hydrodynamic properties of microalgae culture measurement	89
3.6.2	Theoretical framework of mass transfer phenomenon in pilot-scale microalgae culture	90
3.6.3	Experimental validation on mass transfer coefficient	92
3.6.4	Empirical calculation for hydrodynamic parameters	93
3.6.4(a)	Interfacial area	93
3.6.4(b)	Superficial gas velocity	93
3.6.4(c)	Overall gas holdup	94
3.6.4(d)	Bubble rise velocity	94
3.6.4(e)	Average bubble diameter	95
3.6.4(f)	Maximum bubble diameter	95
3.6.5	Mechanism of air bubbles distribution in microalgal culture	95
3.7	Validation analysis on microalgae growth performance	99
3.8	Harvesting of microalgal biomass	100
3.8.1	Flocculation efficiency	100
3.8.2	Characterization of harvesting water	101
3.9	Cultivation of microalgae by using harvesting water	103
3.10	Characterization of microalgal biomass	103
3.10.1	Biochemical analysis	103
3.10.2	Proximate analysis	106
3.10.3	Elemental analysis	107
3.10.4	Functional groups analysis	107
3.10.5	Combustion behavior	107

3.11	Hydrothermal carbonization (HTC)	108
3.11.1	Hydrochar	109
3.11.1(a)	Proximate analysis	110
3.11.1(b)	Elemental analysis	110
3.11.1(c)	Energetic assessment	110
3.11.1(d)	Functional groups analysis	111
3.11.1(e)	Combustion behavior	111
3.11.2	Aqueous phase products	111
3.11.2(a)	Total organic carbon content	112
3.11.2(b)	Nutrients concentration	112
3.11.2(c)	Chemical oxygen demand	112
3.11.2(d)	Cultivation of microalgae via aqueous phase as nutrient source	113
3.12	Reproducibility of the result	113
CHAPTER 4 RESULTS AND DISCUSSION		115
4.1	Optimization of microalgal culturing parameters	115
4.1.1	Effect of inoculum concentration	116
4.1.2	Effect of photoperiod	119
4.1.3	Effect of compressed-air aeration rate	121
4.2	Influences of hydrodynamic stress and gas-liquid mass transfer on microalgae growth	128
4.2.1	Characterization of hydrodynamic stress	128
4.2.2	Characterization of volumetric gas-liquid mass transfer coefficient	130

4.2.3	Correlation between hydrodynamic stress and volumetric gas-liquid mass transfer coefficient	135
4.3	Evaluation on mass transfer influence towards CO ₂ solubility and pH of microalgae cultures	138
4.3.1	Microalgae growth performance at varied CO ₂ concentration	138
4.3.2	Postulation of the CO ₂ solubility mechanism	141
4.3.3	Assessment on CO ₂ solubility performance	144
4.3.4	Influence of CO ₂ solubility towards culturing pH	148
4.3.5	Microalgae growth under acidic conditions	149
4.3.6	Summary	152
4.4	Modelling of microalgal growth	153
4.5	Validation of microalgal growth model	156
4.6	Reusability of water from harvesting process	161
4.7	Microalgal biomass harvesting	166
4.8	Application of microalgal biomass as bio-energy feedstock	173
4.8.1	Characterization of microalgal biomass	173
4.8.2	Hydrothermal carbonization process	180
4.8.3	Qualitative analysis on hydrochar	183
4.8.3(a)	Composition analysis	183
4.8.3(b)	Analysis of functional groups in hydrochar	191
4.8.3(c)	Combustion behaviour of hydrochar	193
4.8.4	Application of hydrothermal carbonization by-product	198
4.8.4(a)	Characterization of aqueous phase	198
4.8.4(b)	Cultivation of microalgae with HTC produced aqueous phase	202

4.9	Summary	205
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CHAPTER 5 CONCLUSION AND RECOMMENDATIONS 208

5.1	Conclusion	208
-----	------------	-----

5.2	Recommendations	209
-----	-----------------	-----

REFERENCES 211

APPENDICES

APPENDIX A: MICROALGAE GROWTH

APPENDIX B: HPLC CALIBRATION FOR CARBOHYDRATE
CONTENT ANALYSIS

APPENDIX C: GAS-LIQUID MASS TRANSFER PHENOMENON

APPENDIX D: STATISTICAL ANALYSIS RESULTS

LIST OF PUBLICATION

LIST OF TABLES

		Page
Table 2.1	Categories of microalgal culturing parameters.	18
Table 2.2	The advantages and drawbacks of different types of photobioreactors for microalgae cultivation. Summarized from (Ugwu et al. (2008), Mata et al. (2010), Zittelli et al. (2013)).	39
Table 2.3	Commonly used kinetic models that incorporate various correlation factors such as inorganic carbon concentration, nitrogen concentration, phosphorus concentration, light intensity, inhibition, temperature, and a combination of multiple factors.	47
Table 2.4	Study on hydrothermal processing of microalgal biomass by using water as a medium without the presence of the catalyst.	57
Table 3.1	List of chemicals and test kits used in this research.	64
Table 3.2	List of equipment used in this research study.	65
Table 3.3	Characterization of organic fertilizer medium used for microalgae <i>Chlorella vulgaris</i> cultivation (Adapted from Lam and Lee (2012)).	71
Table 3.4	Operating parameters of triple quadrupole inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer, NexION® 300).	102
Table 4.1	The average values of DO concentration measured at various conditions throughout 15-day of batch cultivation under various compressed-air aeration rates.	130

Table 4.2	Comparison of average bubble diameter and maximum bubble diameter yielded in BC-PBR microalgae cultivation system.	133
Table 4.3	The carbon content (C_{carbon}), microalgal biomass productivity (P), carbon dioxide biofixation rate (R), and experimental CO ₂ solubility of microalga <i>C. vulgaris</i> under different CO ₂ concentrations and compressed-air aeration rates.	145
Table 4.4	The goodness of fit test for the experimental and simulated data (using the Logistic model) via fitted line plot method for microalgae cultivation in both batch and semi-continuous modes.	155
Table 4.5	The goodness of fit test for the experimental and simulated data (using the modified Logistic model) via the fitted line plot method for microalgae cultivation in semi-continuous mode only.	157
Table 4.6	Comparison of the nutrients consumption for total nitrogen and total phosphorus for control and recycled cultivation mediums under optimum cultivation conditions in a pilot-scale semi-continuous bubble column photobioreactor. Other cultivation conditions: pH = 3, inoculum concentration $\approx 0.3 \text{ g L}^{-1}$, compressed-air aeration rate = 0.16 vvm, and illumination = 24 h with light intensity of $60 - 70 \mu\text{mol m}^{-2} \text{ s}^{-1}$.	163
Table 4.7	Trace metal elements analysis through ICP-MS on the control medium and recycled medium that sampling at the end of semi-continuous cultivation (day 30).	165
Table 4.8	Properties of the dried microalga <i>Chlorella vulgaris</i> biomass.	174
Table 4.9	Characteristic band regions of microalgal biomass in FTIR spectrum.	178

Table 4.10	Process parameters and resulting properties of hydrochar with constant biomass to water ratio (B/W = 0.1). Analyses performed in duplicate with average values to be reported.	184
Table 4.11	Characteristic temperatures of selected hydrochar under HTC retention times of 0.5h as data representative.	196
Table 4.12	Aqueous phase analysis from HTC of microalga <i>Chlorella vulgaris</i> biomass at different hydrothermal temperatures and retention times with constant biomass to water ratio (B/W = 0.1).	198
Table 4.13	Distribution of nutrients parameters sourced from organic fertilizer and HTC produced aqueous phase.	203

LIST OF FIGURES

	Page	
Figure 2.1	Phenomena occurring during microalgae cultivation within photobioreactors: (a) macroscale transport phenomena and (b) microscale kinetic growth for microalgae cells.	16
Figure 2.2	The schematic diagram of a typical photosynthetic-intensity (PI) response curve (i.e., the dependency of microalgae photosynthetic rate on light intensity). Adapted from Béchet et al. (2013).	20
Figure 2.3	Growth phases in photosynthetic microalgae culture	30
Figure 2.4	Semi-continuous culture at quasi steady state, where S and μX represented substrate concentration, biomass output (μ = specific growth rate \times X = biomass concentration), respectively.	31
Figure 2.5	Continuous culture at steady state condition, where X and XD represented biomass concentration and biomass output rate as a functions of dilution rate (D).	32
Figure 2.6	Three major outdoor open cultivation systems for commercial scale (Benemann, 2013): (a) Cultivation of microalga <i>Haematococcus pluvialis</i> (red ponds) and <i>Spirulina</i> in raceway ponds, Cynotech Co., Hawaii; (b) Cultivation of microalga <i>Chlorella</i> in circular ponds, Chlorella Industry, Japan; and (c) Cultivation of microalga <i>Dunaliella</i> in unmixed ponds, Betatene (Cognis), West Australia.	34
Figure 2.7	The empirical kinetic profiles for primary models	42
Figure 2.8	Microalgal biofuels production pathways. Adapted from Brennan and Owende (2010).	53

Figure 2.9	Major biofuel products distribution according to hydrothermal processing conditions by using microalgal biomass. Adapted from Hrnčič et al. (2016).	56
Figure 2.10	Phase diagram of water with an area of application for hydrothermal treatment, i.e. sub- and supercritical water (T _c : Critical temperature, P _c : Critical pressure). Highlighted area indicated regions for supercritical water and subcritical water properties. Adapted from Hrnčič et al. (2016).	58
Figure 3.1	Experimental design.	62
Figure 3.2	Schematic diagram for operating BC-PBR: (a) dimensions of BC-PBR with static liquid flow and (b) illustration of flow regimes in BC-PBR.	67
Figure 3.3	Schematic diagram of hydrothermal carbonization reactor.	69
Figure 3.4	Repeatable growth profile of microalga <i>Chlorella vulgaris</i> in a 5 L photobioreactor during adaption period.	72
Figure 3.5	Optical micrographs on brightfield image for microalgae cells collected from (a) microalgae seed culture; and (b) microalgae cells after adapted for 14 days in 5L PBR cultivation system.	73
Figure 3.6	Microalga <i>Chlorella vulgaris</i> cultivated growth curve for various phases in pilot-scale bubble column photobioreactor: (i) Exponential growth, (ii) Linear growth, and (iii) Stationary growth.	75
Figure 3.7	Comparison of microalgae biomass accumulation for batch and semi-continuous cultivation mode.	76

Figure 3.8	Schematic diagram of air bubbles distribution mechanism in pilot-scale BC-PBR cultivation system for microalga <i>Chlorella vulgaris</i> : (a) Formation of bubbles, (b) Rising of bubbles, and (c) Break up of bubbles	97
Figure 4.1	Growth profiles (mean \pm SE, n = 3) of <i>Chlorella vulgaris</i> cultured at different inoculum concentrations under semi-continuous cultivation mode: (i) batch cultivation linear phase, (ii) first semi-continuous cycle, (iii) second semi-continuous cycle, and (iv) third semi-continuous cycle. Other cultivation conditions: pH = 3, compressed-air aeration rate = 0.16 vvm, and illumination = 24 h with light intensity of 60 – 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$.	117
Figure 4.2	Growth profiles (mean \pm SE, n = 3) of <i>Chlorella vulgaris</i> cultured at different photoperiods under semi-continuous cultivation mode: (i) batch cultivation linear phase, (ii) first semi-continuous cycle, (iii) second semi-continuous cycle, and (iv) third semi-continuous cycle. Other cultivation conditions: pH = 3, inoculum concentration \approx 0.3 g L ⁻¹ , and compressed-air aeration rate = 0.16 vvm.	120
Figure 4.3	Growth profiles (mean \pm SE, n = 3) of <i>Chlorella vulgaris</i> cultured at different compressed-air aeration rate under semi-continuous cultivation mode: (i) batch cultivation linear phase, (ii) first semi-continuous cycle, (iii) second semi-continuous cycle, and (iv) third semi-continuous cycle. Other cultivation conditions: pH = 3, inoculum concentration \approx 0.3 g L ⁻¹ , and illumination = 24 h with light intensity of 60 – 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$.	123

- Figure 4.4 TEM micrographs showing the destruction of cell wall on microalga *Chlorella vulgaris* surface collected from 0.19 vvm cultivation condition. The microalgae cells that performed continuously growth under optimized semi-continuous cultivation: (a) Active cell sample – undamaged cell with a rigid cell wall around microalgae collected by day-15 of cultivation. The microalgae cells at death event (b and c: inactive cells samples) under third semi-continuous cycle at 0.19 vvm aeration rate by day-30 cultivation; (b) damaged cell with disintegrated cell wall and (c) damaged cells with disintegrated of cell boundaries that led to destruction in cell morphology. 125
- Figure 4.5 Rheology analysis for microalgae *Chlorella vulgaris* culture at ambient temperature throughout different microalgal cultivation stages (for a total of 30 days semi-continuous cultivation under various compressed-air aeration rate) in pilot-scale BC-PBR. Trend line: (a) Plot of shear stress versus shear rate; and (b) Plot of viscosity versus shear rate. Data reported are in average value. 129
- Figure 4.6 The average microalgae biomass dry weight accumulation with its respective experimental volumetric mass transfer coefficients for the 30 days of semi-continuous cultivation under optimum culturing parameters. Other cultivation conditions: pH = 3, inoculum concentration $\approx 0.3 \text{ g L}^{-1}$, and illumination = 24 h with light intensity of $60 - 70 \mu\text{mol m}^{-2} \text{ s}^{-1}$. 132

- Figure 4.7 Effects of hydrodynamic stress towards experimental gas-liquid mass transfer coefficient towards the changing in average of $k_{LaL}(\text{CO}_2)/\varepsilon_G$ ratio and average of $k_{LaL}(\text{CO}_2)/a_L$ ratio, with respect to the individual superficial gas velocity, U_G that measured at different compressed-air aeration rates (0.12, 0.14, 0.16, 0.18 and 0.19 vvm) provided to microalgal cultivation system in bubble column photobioreactor throughout 30 days of semi-continuous cultivation. Other cultivation conditions: pH = 3, inoculum concentration $\approx 0.3 \text{ g L}^{-1}$, and illumination = 24 h with light intensity of $60 - 70 \mu\text{mol m}^{-2} \text{ s}^{-1}$. 136
- Figure 4.8 Growth profiles (mean \pm SE, n = 3) of *Chlorella vulgaris* cultured at different CO_2 concentration under semi-continuous cultivation mode: (i) batch cultivation linear phase, (ii) first semi-continuous cycle, (iii) second semi-continuous cycle, and (iv) third semi-continuous cycle. Other cultivation conditions: pH = 3, inoculum concentration $\approx 0.3 \text{ g L}^{-1}$, compressed air aeration rate = 0.16 vvm, and illumination = 24 h with light intensity of $60 - 70 \mu\text{mol m}^{-2} \text{ s}^{-1}$. 139
- Figure 4.9 Growth profiles (mean \pm SE, n = 3) of *Chlorella vulgaris* cultured at different initial culture pH under semi-continuous cultivation mode: (i) batch cultivation linear phase, (ii) first semi-continuous cycle, (iii) second semi-continuous cycle, and (iv) third semi-continuous cycle. Other cultivation conditions: Inoculum concentration $\approx 0.3 \text{ g L}^{-1}$, compressed-air aeration rate = 0.16 vvm, and illumination = 24 h with light intensity of $60 - 70 \mu\text{mol m}^{-2} \text{ s}^{-1}$. 150

- Figure 4.10 Comparison of experimental and predicted values (using the modified Logistic model) of microalgae biomass dry weight via semi-continuous cultivation mode in the designated BC-PBRs for selected compressed-air aeration rates as data representative. 157
- Figure 4.11 Validation analysis of growth profiles (mean \pm SE, n = 3) of *Chlorella vulgaris* cultivated in pilot-scale bubble column photobioreactor (56 L BC-PBR) for 120 days (or 21 semi-continuous cycles): (a) Comparison of experimental and predicted microalgal biomass production, (b) Microalgae growth profiles for extended semi-continuous growth, and (c) Microalgal biomass accumulation for each semi-continuous cycle. Cultivation conditions: Inoculum concentration \approx 0.3 g L⁻¹, supplied with compressed air aeration rate of 0.16 vvm, illumination continuously for 24 h with light intensity of 60 – 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and culture was maintained at pH 3. 159
- Figure 4.12 Growth profiles (mean \pm SE, n = 3) of *Chlorella vulgaris* in control and recycled medium under semi-continuous cultivation mode: (i) batch cultivation linear phase, (ii) first semi-continuous cycle, (iii) second semi-continuous cycle, and (iv) third semi-continuous cycle. Other cultivation conditions: inoculum concentration \approx 0.3 g L⁻¹, compressed-air aeration rate = 0.16 vvm, and illumination = 24 h with light intensity of 60 – 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 162
- Figure 4.13 Performance of microalgae harvesting through the flocculation process by using Alum as flocculant : (a) Optimization of flocculation pH and (b) Optimization of the flocculant dosage. 169

Figure 4.14	The pC-pH diagram for aluminium ion, $Al^{3+} = 1.85 \times 10^{-9}$ M (Jensen, 2003).	172
Figure 4.15	FTIR spectrum of microalgal biomass.	177
Figure 4.16	The TG-DTG profile of microalgal biomass in an air atmosphere.	179
	Note: Stage A represented weight loss from evaporation of water, Stage B represented the release of volatiles and combustion, Stage C represented the combustion of volatiles, and Stage D represented the passive combustion reaction zone.	
Figure 4.17	Hydrochar yield from HTC of microalga <i>Chlorella vulgaris</i> biomass at different hydrothermal temperatures and retention times with constant biomass to water ratio (B/W = 0.1)	182
Figure 4.18	Carbon densification of hydrochar produced from HTC of microalga <i>Chlorella vulgaris</i> biomass at different hydrothermal temperatures and retention times with constant biomass to water ratio (B/W = 0.1).	188
Figure 4.19	The van Krevelen diagram of hydrochar produced from microalgal biomass under various HTC processing conditions.	189
Figure 4.20	FTIR spectra of microalgal raw biomass and selected hydrochars processing at hydrothermal temperatures 200, 210 and 220 °C for a retention time of 0.5 h. Note for regions: 1: -OH, 2: -CH ₂ and -CH ₃ , 3: -CH ₂ and -CH ₃ , 4: C=O and N-H, 5: C=O and N-H, 6: C-O-R, 7: C-O.	192

- Figure 4.21 TG-DTG thermogram for combustion of microalgal raw biomass and selected hydrochar samples ($T = 200, 210 \text{ \& } 220 \text{ } ^\circ\text{C}$) under HTC retention times of 0.5 h as data representative: (a) TG profile and (b) DTG profile. 194
- Note: Stage A & A1 represented weight loss from evaporation of water, Stage B & B1 represented the release of volatiles and combustion, Stage C & C1 represented the combustion of volatiles, and Stage D represented the passive combustion reaction zone.
- Figure 4.22 The pH of the aqueous phase separated after HTC of microalga *Chlorella vulgaris* biomass at different hydrothermal temperatures and retention times with constant biomass to water ratio ($B/W = 0.1$). 200
- Figure 4.23 The COD/TOC ratios of aqueous phase separated after HTC of microalga *Chlorella vulgaris* biomass at different hydrothermal temperatures and retention times with constant biomass to water ratio ($B/W = 0.1$). 202
- Figure 4.24 Comparison of growth performances of microalga *Chlorella vulgaris* that utilized HTC produced aqueous phase as nutrient source with the control sample for semi-continuous cultivation mode: (i) batch cultivation linear phase, (ii) first semi-continuous cycle, (iii) second semi-continuous cycle, and (iv) third semi-continuous cycle. Other cultivation condition: inoculum concentration $\approx 0.3 \text{ g L}^{-1}$, illumination = 24 h with light intensity of $60 - 70 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$, compressed-air aeration rate = 0.16 vvm, and pH 3. 204

LIST OF PLATE

	Page
Plate 4.1	167
<p>Microalgal biomass harvesting process through flocculation method: (a) 15 L of microalgae culture harvested from BC-PBR cultivation system, (b) Addition of flocculants into microalgae culture under optimum flocculation conditions, (c) Formation of larger floc that would settled down in microalgae culture, (d) Two distinguished layers were observed, with supernatant as water and microalgae floc at the bottom layer, (e) The supernatant was decanted while microalgae flocs were collected as wet biomass, and (f) Dried microalgal biomass.</p>	

LIST OF SYMBOLS

Symbol	Description	Unit
a	y-intercept of plot t versus $\ln [(K/N)-1]$	Dimensionless
a_L	Interfacial area per unit volume	m^{-1}
A	BC-PBR aeration cross-sectional area	m^2
C	Dissolved Oxygen (DO) concentration	%
C^*	Saturated Dissolved Oxygen (DO) concentration	%
C_0	Initial Dissolved Oxygen (DO) concentration	%
C_{carbon}	Carbon content	w/w
d_b	Average bubble diameter	mm
$d_{b, \text{max}}$	Maximum bubble diameter	mm
d_0	Diameter of gas sparger hole	mm
D	Diffusivity	$\text{m}^2 \text{s}^{-1}$
D_{O_2}	Diffusivity of oxygen, O_2 at 298 K	$\text{m}^2 \text{s}^{-1}$
D_{CO_2}	Diffusivity of carbon dioxide, CO_2 at 298 K	$\text{m}^2 \text{s}^{-1}$
D_R	Inner diameter of BC-PBR	m
g	Gravitational acceleration	m s^{-2}
H	Henry's constant	atm/M
H_c	Height of sample collection pot	m
H_F	Height of static fluid	m
H_{GL}	Height of aerated gas-liquid dispersion phase	m
H_R	Height of BC-PBR	m
K	Maximum biomass density	g/L
k_L	Liquid phase mass transfer coefficient	m s^{-1}

Symbol	Description	Unit
k_{GA_L}	Volumetric gas phase mass transfer coefficient	s^{-1}
K_{LA_L}	Overall volumetric gas-liquid mass transfer	s^{-1}
k_{LA_L}	Volumetric liquid phase mass transfer coefficient	s^{-1}
K	Solubility products	
K_H	Henry;s Law constant	Dimensionless
$K_{H_2CO_3}^\circ$	Solubility constant of carbonic acid	Dimensionless
$K_{HCO_3^-}^\circ$	Solubility constant of HCO_3^-	Dimensionless
K_w°	Solubility constant of water	Dimensionless
LPM	Unit for compressed air aeration flow rate	$L \text{ min}^{-1}$
N	Microalgal biomass density	g/L
OD_{540}	Optical density at wavelength of 540 nm	Dimensionless
P	Microalgal biomass productivity	$mg \text{ L}^{-1} \text{ d}^{-1}$
P_G	Specific power input due to BC-PBR aeration	W
Q_{GL}	Volume of aerated gas-liquid dispersion phase	L
Q_L	Volume of unaerated microalgae culture	L
r	Gradient of plot t versus $\ln [(K/N)-1]$	Dimensionless
R	CO_2 bio-fixation rate	$mg \text{ CO}_2 \text{ L}^{-1} \text{ d}^{-1}$
Re_G	Reynolds number in gas phase	Dimensionless
Re_0	Reynolds number from gas sparger	Dimensionless
t	Cultivation time	day
t_d	Microalgae cells doubling time	day
t_R	Thickness of BC-PBR	mm
T_i	Ignition temperature	$^\circ\text{C}$
T_b	Burnout temperature	$^\circ\text{C}$

Symbol	Description	Unit
U_b	Bubble rise velocity	m s^{-1}
U_G	Superficial gas velocity	m s^{-1}
V_L	Liquid velocity of microalgae culture	m s^{-1}
vvm	Unit of aeration rate that correlated between gas input and reactor volume – volume of gas (L) per unit volume of liquid (L) per minute (min)	$\text{L L}^{-1} \text{min}^{-1}$
$x_{\text{CO}_2}^*$	CO ₂ equilibrium mole fraction in solution	mol
$y_{\text{CO}_2}^*$	CO ₂ equilibrium mole fraction in the gas phase	mol
$y_{\text{CO}_2}^{*'}$	Experimental CO ₂ mole fraction in the gas phase	mol
ε_G	Overall gas holdup	Dimensionless
η	Flocculation efficiency	%
μ_L	Viscosity of microalgae culture	cP
μ_G	Viscosity of bubbles swarm	cP
μ_{max}	Maximum specific growth rate	day^{-1}
ρ_L	Density of microalgae culture	kg/m^3
ρ_G	Density of swarm of bubbles	kg/m^3
σ	Surface tension	Dynes/cm
τ	Shear stress of microalgae culture	D/cm^2
γ	Shear rate of microalgae culture	1/sec
Δ	A change in the value of a variable in calculus	Dimensionless

LIST OF ABBREVIATIONS

Alum	Aluminium sulphate 16-Hydrate
BC-PBR	Bubble column photobioreactor
B/W	Biomass to water ratio
CO ₂	Carbon dioxide
COD	Chemical oxygen demand
DTG	Derivative thermogravimetry
EY	Energy yield
FC	Fixed carbon
FT-IR	Fourier transform infrared spectrometer
HHV	Higher heating value
HPLC	High performance liquid chromatography
HTC	Hydrothermal carbonization
ICP-MS	Inductively coupled plasma mass spectrometry
O ₂	Oxygen gas
N ₂	Nitrogen gas
TG	Thermogravimetry
TN	Total nitrogen
TP	Total phosphorus
TOC	Total organic carbon
VM	Volatile matter

PENGARUH PEMINDAHAN JISIM TERHADAP PENGKULTURAN
***Chlorella vulgaris* DI DALAM FOTOBIOREAKTOR TURUS GELEMBUNG**
SECARA SEPARA BERTERUSAN

ABSTRAK

Mikroalga, terkenal dengan kecekapan fotosintesis dan pertumbuhan yang tinggi muncul sebagai bahan mentah untuk menghasilkan bio-tenaga generasi ketiga. Dalam kajian ini, *Chlorella vulgaris* telah dipilih sebagai subjek penyelidikan. Tujuan projek penyelidikan ini adalah untuk memaksimumkan pengeluaran biojisim dengan melingkupi kinetik dan fenomena pemindahan jisim semasa pengkulturan *Chlorella vulgaris* dalam skala perintis. Untuk mencapai maksimum pengeluaran biojisim, keadaan pengkulturan mikroalga secara separa berterusan telah dioptimumkan. Setiap kitaran pengkulturan mikroalga melibatkan 15 hari mod kelompok dan diikuti dengan 3 kitaran mod separa berterusan (5 hari untuk setiap kitaran). Kaedah satu-faktor-pada-satu-masa (OFAT) digunakan untuk menyiasat kesan kepekatan inokulum, tempoh pencahayaan, dan kadar pengudaraan terhadap kadar pertumbuhan mikroalga dalam lingkungan masing-masing 0.10 – 0.35 g L⁻¹, 12 dan 24 j, dan 0.12 – 0.19 vvm. Mekanisma pemindahan jisim di antara gas karbon dioksida (CO₂) dengan media kultur turut dikaji pada keadaan pengkulturan optimum. Tambahan lagi, potensi penggunaan air kitar semula dari pengkulturan selepas proses pemberbukuan turut dinilai. Bagi kajian penggunaan biojisim mikroalga sebagai bio-tenanga, biojisim mikroalga yang terkumpul kemudian ditukarkan kepada hidrochar melalui proses tindakbalas karbonisasi hidrotermal (HTC). Kesan suhu dan masa tindakbalas HTC terhadap ciri hidrochar yang dihasilkan telah dikaji pada julat 180 – 250 °C dan 0.5 – 4 jam masing-masing. Kajian ini menunjukkan pengeluaran biojisim mikroalga

maksimum iaitu 0.9819 g L^{-1} , diperolehi pada keadaan pengkulturan berikut; kepekatan inokulum 0.30 g L^{-1} , pencahayaan berterusan (24 j) dengan keamatan cahaya $60 - 70 \mu\text{mol m}^{-2} \text{ s}^{-1}$, dan kadar pengudaraan 0.16 vvm . Pekali pemindahan jisim gas-cecair, $k_{LaL}(\text{CO}_2)$ didapati adalah 0.45 s^{-1} , dan didapati sistem pengkulturan melalui mekanisma pemisahan gelembung semasa mengangkut proses pemindahan gas CO_2 ke dalam media kultur. Peranan pemindahan jisim dalam pertumbuhan mikroalga telah turut dikaji dengan meningkatkan kepekatan gas CO_2 yang dibekalkan kepada sistem pengkulturan BC-PBR. Berdasarkan keputusan yang diperolehi, peningkatan dalam pengumpulan biojisim didapati tidak dipengaruhi oleh kenaikan kepekatan gas CO_2 . Ini adalah disebabkan oleh keterbatasan kelarutan CO_2 ke dalam kultur media. Seterusnya, satu model matematik berdasarkan keadaan pertumbuhan mikroalga optimum dalam BC-PBR skala perintis telah dibangunkan untuk meramalkan kadar penghasilan biojisim. Penggabungan parameter pemindahan jisim dalam model pertumbuhan yang berubah-suai telah disahkan dengan melanjutkan tempoh pengkulturan separa berterusan kepada 120 hari (21 kitaran). Keputusan yang diperolehi menunjukkan bahawa mikroalga dapat tumbuh dalam air kultur media yang dikitar semula. Selain itu, keputusan eksperimen mendapati bahawa hidrochar dengan hasil tenaga tertinggi pada 76.59% boleh diperolehi melalui proses HTC pada $210 \text{ }^\circ\text{C}$ dan 0.5 jam . Nilai HHV bagi hidrochar yang dihasilkan didapati adalah 24.51 kJ g^{-1} berbanding biojisim mentah pada 12.58 kJ g^{-1} . Selain itu, proses HTC didapati menghasilkan fasa cecair yang boleh digunakan sebagai sumber nutrisi alternatif bagi pengkulturan mikroalga, dengan nilai purata hasil penghasilan biojisim 0.8483 g L^{-1} . Kesimpulannya, pemindahan jisim adalah faktor dominan yang mempengaruhi kinetik pengkulturan mikroalga dalam sistem pengkulturan BC-PBR secara separa berterusan pada skala perintis. Seterusnya, ia mempengaruhi kualiti biojisim yang dihasilkan, dan

mempengaruhi laluan pemprosesan yang dipilih untuk penukaran biotenaga yang optimum.

INFLUENCE OF MASS TRANSFER TOWARDS PILOT-SCALE SEMI-CONTINUOUS CULTIVATION OF *Chlorella vulgaris*

ABSTRACT

Microalgae, well-known for their prominent photosynthetic efficiency and rapid growth rate emerge as a great feedstock for bio-energy production of third-generation biofuel. In this study, *Chlorella vulgaris* was chosen as the subject of investigation. The aim was to maximize the biomass production by investigating both the kinetic and mass transfer phenomena in a pilot-scale bubble column photobioreactor (BC-PBR) cultivation system. To account for the maximum microalgal biomass accumulation, the microalgae growth condition was optimized in the semi-continuous cultivation mode. Each cultivation cycle was carried out with 15 days of batch cultivation mode, followed by 3 cycles of 5 days each during semi-continuous cultivation mode. One-factor-at-a-time (OFAT) method was employed to investigate the effects of inoculum concentration of microalgae cells, photoperiod, and aeration rate towards microalgal growth performance, in the range of 0.1 – 0.35 g L⁻¹, 12 and 24 h, and 0.12 – 0.19 vvm, respectively. The underlying mass transfer mechanism between gaseous CO₂ and the culture medium were investigated under the optimized growth conditions. In addition, the reusability of the recycled water from the harvesting process was evaluated. To convert the microalgae into application biofuel, the harvested microalgal biomass was then converted into hydrochar via hydrothermal carbonization (HTC) reaction. The effects of hydrothermal temperature and retention time and the properties of hydrochar were studied at the range of 180 – 250 °C and 0.5 – 4 h, respectively. The research results showed that the optimum biomass accumulation was at 0.9819 g L⁻¹, with cultivation conditions of: inoculum

concentration of 0.3 g L^{-1} , exposed under continuous (24 h) illumination with light intensity $60 - 70 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$, and supplied with compressed air at aeration rate of 0.16 vvm. The cultivation system underwent a bubble breakup mechanism during the transportation of gaseous CO_2 into the culture medium with gas-liquid mass transfer coefficient, $k_{Lal}(\text{CO}_2)$ of 0.45 s^{-1} . Higher CO_2 concentration environment did not affect the biomass accumulation due to the solubility limitation of CO_2 in the microalgae culture. Based on optimized growth conditions for microalgae, a mathematical model for microalgae growth was developed. By incorporating the mass transfer parameter into the modified growth model, which was validated through an extended 120 days (21 cycles) of semi-continuous cultivation. In addition, the microalgae cells were proven to be able to grow in the recycled harvesting water. On the other hand, the highest energy yield of hydrochar was achieved at 76.59%, at the HTC under $210 \text{ }^\circ\text{C}$ for 0.5 h. Comparatively, higher heating value (HHV) of hydrochar produced was measured to be 24.51 kJ g^{-1} , which is higher than that of raw biomass (12.58 kJ g^{-1}). Moreover, the HTC process produced an aqueous phase that could be used as an alternative nutrient source for microalgae cultivation, yielding an average biomass accumulation of 0.8483 g L^{-1} , demonstrating the feasibility of close loop cultivation. To conclude, mass transfer was a dominant factor affecting the kinetic growth of microalgae in pilot-scale semi-continuous BC-PBR cultivation system. It further affected the quality of produced biomass, and thus affected the downstream processing route chosen for optimal conversion of bioenergy.

CHAPTER 1

INTRODUCTION

This chapter provides an introductory on the biofuels development from first generation to third generation microalgal biofuels. The issues of energy security and environmental sustainability are discussed. The research problem statement is being discussed, while the objectives and scopes of the study are being devised accordingly. Lastly, the thesis organization is given in the last section.

1.1 Background of study

There are two major challenges confronting humanity globally, which are environmental sustainability and energy crises due to overly dependent on fossil fuels. The immense consumption of fossil fuels has resulted in global warming owing to the huge emission of greenhouse gases (GHGs) into the atmosphere. Therefore, developing alternative renewable energy resources has emerged as a priority to many researchers worldwide.

A negative impact of the food versus fuels debate has arisen due to the use of first-generation renewable energy derived from edible feedstocks such as sugarcane and corn (Gui et al., 2008). This led to the development of second-generation renewable energy derived from lignocellulosic biomass feedstocks such as forest and agricultural residues. However, owing to the complex structure of lignocellulosic biomass, pretreatment of biomass and cost-intensive recovery are required for the subsequent biofuel production (Limayem & Ricke, 2012). Hence, one of the possible solutions to these problems is to use microalgae as third-generation renewable energy.

The growth of algae is sustained through the photosynthesis process, which allows it to play its role as the primary producer in aquatic environments. Algae colonized various habitats such as marine and freshwater, meanwhile capable to develop a symbiotic relationship with other organisms (Andersen, 2013). Algae is widely used as alternative bioenergy resources, with a photosynthetic characteristic that requires light, sugars, carbon dioxide (CO₂), nitrogen (N), phosphorus (P) and potassium (K) for growing (Brennan and Owende, 2010). Owing to its diversified biochemical compositions (i.e. carbohydrates, lipids, and proteins), algal biomass can produce a wide range of commercially valuable bioproducts (Barsanti and Gualtieri, 2014). Besides that, growing algae cells with wastewater and flue gas could further enhanced the environmental sustainability of algal biomass production (Ahmad et al., 2011).

Recently, microalgae have been intensively investigated as a technically viable and sustainable bioenergies and bioproducts production through downstream biorefineries. Considering the limited availability of land area, algae-based biofuels production, which includes biochar, biodiesel, bio-oil, biosyngas, and bio-hydrogen are possible to satisfy the fast-growing energy demand (Li et al., 2008). Several technological developments in this research area such as photobioreactor design, microalgal biomass harvesting and drying, integrated microalgal farming and biorefinery strategy of biofuels, have enhanced the cost-effectiveness of biofuels production (Brennan & Owende, 2010). Additionally, high-value commodities such as vitamins, lipids, pigments etc. could be extracted from microalgal biomass. The integration of bioenergies and bioproducts processing has led towards a step closer for commercial implementation of microalgae-based biofuels, as a feasible alternative renewable energy resource. Microalgae species from the family of *Chlorella* and

Scenedesmus were most commonly used for the production of biofuels (e.g. biodiesel, bioethanol, bio-oil and biochar) (Biller and Ross, 2011) and bioproducts (e.g. carotenoid, omega-3 fatty acids and glycerin) (Batista et al., 2013).

1.2 Problem statement

Microalgae, conventionally defined as unicellular and simple multi-cellular photosynthetic microorganisms, are the most important primary producer of biomass in the aquatic biome. Microalgae have also been used in many different fields, such as CO₂ sequestration from the atmosphere or flue gases (Kumar et al., 2014), and wastewater treatment (Hwang et al., 2016). Additionally, it is also used for the production of high value products such as human health foods (Batista et al., 2013), animal feeds, fish foods (Byreddy et al., 2019), natural pigments (Begum et al., 2016), and pharmaceutical compounds (Mimouni et al., 2012). In particular, some microalgae are rich in lipids, which can be utilized as a feedstock for biofuel production (Brennan and Owende, 2010).

Despite of the great potential of microalgal biofuel production and the enormous technology advances, obstacles, such as the high cost and energy intensity of microalgal farming need to be overcome before commercialization of microalgal biofuel production to be economically viable (Slade and Bauen, 2013). The factors causing high cost include the biomass loss, which results from contamination (Peng et al., 2016) and detrimental effects of oxygen stress that suppressed the biomass productivity (Peng et al., 2013). Cutting down the cost of microalgal cultivation can be potentially achieved by eliminating the costly sterilization process, providing better

process control and deoxygenation through an efficient aeration system, in order to obtain high biomass concentration and less oxygen accumulation.

Closed photobioreactor system such as photobioreactor (PBR) could be sterilized using filtration, steam, ethanol, or chemical additives to minimize the biological contamination (Wang et al., 2013). However, the sterilization process consumes large quantities of energy. Moreover, the maintenance of sterility at an industrial-scale is very difficult and costly. As a result, commercial-scale microalgal farming using sterile closed PBRs have been limited to production of high value products such as healthy food, pharmaceuticals, and cosmetics. Any simplified cultivation which allows the use of simple cultivation system and easy operation, while harboring lower contamination risk would be welcomed to make microalgal technology more economical appealing (Fishman et al., 2010).

Extensive studies have carried out on improvement of microalgal strain to achieve high biomass concentration in microalgal cultures through simplified cultivation. For example, improvement of microalgal strains through upstream technologies such as strain selection and genetic modification (Rodolfi et al., 2009), and downstream technologies like medium composition optimization (Kanaga et al., 2016). Besides that, process control improvement on protozoan contamination control (Bartley et al., 2013, Peng et al., 2016); process optimization on culturing parameters such as light utilization, oxygen accumulation mitigation, CO₂ fixation, pH control etc. (Cheng et al., 2013, Rai et al., 2015, Baer et al., 2016, Wu et al., 2017)); and improvement of the design of cultivation system (Narala et al., 2016).

The feasibility of microalgae cultivation for biofuels production has been reported in several laboratory-scale studies (Chisti, 2007, Amin, 2009, Ahmad et al.,

2011). However, there are still many aspects that require further development before the production of microalgal biofuels can be commercialized. In previous studies, freshwater green microalga *Chlorella vulgaris* has been established as a promising candidate for lipid production (Lam and Lee, 2012). It is an ideal feedstock for biodiesel production owing to its high triglyceride cell content where most of its fatty acids are saturated fatty acid in the range of 16-20 carbons (Lam and Lee, 2013). However, the main obstacle for pilot-scale microalgae cultivation was the difficulty in maintaining the microalgal biomass productivity at the maximum level as compared to laboratory scale. This was due to the inadequate knowledge on the influences from the complex combination of both kinetic and mass transfer phenomenon took place in the pilot-scale cultivation system.

Many efforts have been made to scale up the cultivation of microalgae for the production of biofuel, but sustaining a large scale cultivation still remains a challenge. One of the major problem that hindered mass production of microalgal biomass is the understanding of influences from environmental stresses such as hydrodynamic stress and gas-liquid mass transfer phenomenon exerted onto large-scale microalgae cultivation system. It is important to understand the underlying mechanisms of the environmental stresses exerted on both microalgae cells and cultivation system at the macroscopic level, in order to enhance the microalgae growth performance. Additionally, cultivation strategies also played an important role in sustaining the growth of microalgae cells in order to enhance the biomass productivity rate.

In terms of optimizing microalgal growth in pilot-scale cultivation system, the kinetic and mass transfer are both two important factors. However, the research on the influences from the gas-liquid mass transfer phenomenon and hydrodynamic stress

exerted on microalgae cultivation system is still lacking in the literature. Additionally, by optimizing the biological and physiological growth parameters on microalgae cells at the microscopic level in a closed photobioreactor (PBR) with a controlled environment (i.e. minimized contamination) could enhance higher microalgal biomass productivity (Masojídek and Torzillo, 2008). All these aspects could contribute to a certain degree of prediction accuracy in optimizing large scale microalgae biomass production. Hence, to address the gap of knowledge, it is worthy to investigate the growth optimization conditions required for pilot-scale cultivation of microalgae that incorporated both kinetic and mass transfer (i.e. macro-and-microscopic levels).

Apart from that, there are not many studies that focused on developing a suitable mathematical model that integrated both gas-liquid mass transfer and kinetically characteristics for microalgae growth performance, especially in pilot-scale microalgae cultivation system. Currently, most of the studies are reported on kinetics models that represented the laboratory cultivation scale. Inadequate knowledge on the phenomenon in pilot-scale microalgae cultivation system would significantly affect the growth rate of microalgae. Hence, it is vital to develop a comprehensive model which considers both kinetic and mass transfer, and hopefully would provide useful insight for future pilot-scale cultivation studies.

Lastly, the high water content in microalgae poses another challenge for the conversion of microalgae to biofuel. One possible solution is to use a hydrothermal treatment route as the water can act as a reaction medium by itself. Through the hydrothermal carbonization process, microalgal biomass can preserve higher energy densification value. It will be interesting to investigate the production of hydrochar through hydrothermal treatment as potential solid biofuel. In addition, the aqueous

phase (by-product) collected can be further analyzed for nutrient recycling during cultivation process, to justify the feasibility of closed loop cultivation.

1.3 Research objectives

The aim of this research is to find an efficient way to control the growth of pilot-scale microalgae, for sustaining the maximum productivity of biomass. The mass transfer resistance was regarded as the manipulating variable. Along with this, we investigate the gas-liquid mass transfer mechanism between the bubbled air and microalgae cells in the pilot-scale cultivation system. The biofuel quality produced from biomass obtained was also carefully analysed as the responding factor. In order to achieve the above stated aim of research, a few objectives were outlined:

1. To optimize the microalgae cultivation conditions in the designated pilot-scale bubble column photobioreactor (BC-PBR) cultivation system with the selected cultivation strategy.
2. To identify the underlying mechanisms of the gas-liquid mass transfer phenomenon with its respective hydrodynamic stress in a pilot-scale semi-continuous cultivation of microalgae cells in a BC-PBR.
3. To generate and validate a suitable microalgae growth model that integrated both mass transfer and kinetic parameters for the pilot-scale semi-continuous cultivation of microalgae in BC-PBR.

4. To verify and demonstrate the application of microalgal biomass as solid fuel (hydrochar) and utilization of biofuel by-product for nutrients recycling through closed loop cultivation process.

1.4 Scopes of study

In this study, the growth performance of microalgae cells in a pilot-scale photobioreactor was studied. Various cultivation factors of microalgae growth were investigated, which included inoculum concentration, illumination period, and aeration rate in order to obtain the optimum growth conditions for maximum biomass production.

In order to investigate the mass transfer, a robust microalga strain is required. Hence, the classical species – *Chlorella vulgaris*, which has wide temperatures tolerance and can grow in harsh environment was selected (Song et al., 2008). Also, *C. vulgaris* was reported to have higher CO₂ fixation rate, which would not hinder the mass transfer results since the ability of cells to utilize maximum amount of CO₂ that dissolved in microalgae culture (in the case for atmospheric CO₂ concentration (0.04%)). For the microalgae cultivation system, the closed system was chosen due to its advantages in the process of controlling and monitoring for microalgae cells, besides being easy in contamination control. The bubble column type photobioreactor was chosen based on the requirement to study mass transfer phenomenon: (1) high gas-liquid transfer and (2) uniform mixing. For the cultivation process, semi-continuous mode was selected in order to increase the microalgal biomass productivity.

In terms of engineering perspective, mathematic modelling plays an important role in optimizing the microalgae growth performance in BC-PBR. A new mathematical model that is aimed to provide an insight into the combined effects between the microalgae growth kinetic and transport phenomena within the BC-PBR cultivation system was developed based on experimental validation results.

The hydrothermal carbonization (HTC) reaction was conducted at hydrothermal temperature for subcritical water range from 180 to 250 °C with reaction time from 0.5 to 4 h. The yield of hydrochar was investigated and characterized according to standard procedures. The combustion behaviour of hydrochar from high-ash content microalgal biomass was analysed in order to investigate the energy densification performance as solid biofuel. In addition, the HTC aqueous phase by-product was investigated for its feasibility as an alternative nutrients source for microalgae growth.

1.5 Thesis organization

This thesis consists of five chapters. Chapter 1 gives an outline of the current global energy scenario, the development of renewable energy, and energy scenario in Malaysia. The problem statement illustrates the problems faced and the need for carrying out the current research study. Apart from that, the research objectives state the aims and purposes of this study. The scope of study elaborates the focus and limitations of this study. Finally, the thesis organization provides a brief information on the content of every chapter in this thesis.

Chapter 2 compiled all literature reviews conducted which include the characteristics of microalgae and culturing parameters, microalgae mass cultivation system design available, challenges in microalgae scale-up cultivation, and the growth performance of microalgae based on mass transfer and growth kinetics. Updated literature covering the application of microalgal biomass for subsequent biofuels production are also presented.

Chapter 3 presents the experimental methodology and analysis. The research design and approach chosen are discussed in detail. Also, the chemicals and materials used throughout the study are listed. Besides, this chapter also provides step by step experimental set-up, microalgae cultivation methods, microalgae growth modelling validation, microalgal biomass and hydrochar analysis and characterization methods.

Chapter 4 presents the results and discussion based on the proposed problem statement. Firstly, the optimization of microalgal biomass production in the pilot-scale bubble column photobioreactor (BC-PBR) was conducted. It was followed by understanding the underlying hydrodynamic stress and mass transfer mechanism in microalgae cultivation in pilot-scale BC-PBR. Then, a new integration growth model that coupled with mass transfer and growth kinetic was developed and further validated through prolonged semi-continuous cultivation. Lastly, the biomass was harvested and characterized for the subsequent hydrochar production via hydrothermal reaction.

Chapter 5 provides a summary of the results obtained in this study. The concluding remarks for each measurable objective are outlined, along with recommendations for future works.

CHAPTER 2

LITERATURE REVIEWS

This chapter conveys concise literature reviews on microalgae cultivation for biomass accumulation. The microalgae growth parameters and various cultivation modes are introduced, especially for large-scale cultivation. Besides that, the challenges facing in microalgae scale-up cultivation was also presented. This is followed by a critical review on the microalgae growth kinetics, which integrates the microalgae cells growth with both mass transfer phenomenon and hydrodynamics effects in large-scale cultivation system (i.e., in a closed photobioreactor). The information presented will give a deeper understanding of the microalgae growth mechanism that can be represented using mathematical modelling. Lastly, hydrothermal processing on microalgal biomass is reviewed for its feasibility as energy storage for advanced biofuels development.

2.1 Microalgae

Microalgae are microscopic and unicellular eukaryotic oxygenic photoautotrophs with a size distribution from 5 to 50 μm (Muylaert et al., 2015). The typical biochemical composition microalgae are carbohydrates, lipids, and proteins that can be converted into various types of biofuels. The carbohydrates can be broken down into monosaccharides through pre-treatment and hydrolysis processes. Depending on the microalgae species and cultivation conditions, different hydrolysis and fermentation (i.e. separate hydrolysis and fermentation, SSF; and simultaneous saccharification and fermentation, SHF) approaches are introduced for bioethanol (Harun et al., 2011). Then, the produced monosaccharides (e.g., glucose) can be

converted to bioethanol through a fermentation process with the addition of suitable yeast strain (John et al., 2011). Microalgae such as *Chlorella* (22–26 wt%), *Chlamydomonas* (50–60 wt%), *Dunaliella* (52wt%), and *Scenedesmus* (45–52 wt%) consist of high carbohydrates content, which is suitable to be cultivated for bioethanol production (Harun et al., 2014).

On the other hand, lipids can be extracted from microalgae to be converted to biodiesel via transesterification process with or without the addition of catalyst. Chemical solvents such as n-hexane, methanol, ethanol and mixed polar/non-polar chemical solvents (e.g., methanol/chloroform and hexane/isopropanol) are effective for extraction of microalgal lipids, but the extraction efficiency is highly dependent on the microalgae strains (Halim et al., 2012). According to a review by Mata et al. (2010), marine and freshwater microalgae species that consist of high lipids content are *Botryococcus braunii* (25–75 wt%), *Chlorella* (18–57 wt%), *Dunaliella tertiolecta* (16.7–71.0 wt%), *Isochrysis galbana* (7–40 wt%), *Nannochloris* sp. (20–56 wt%), *Phaeodactylum tricorutum* (18–57 wt%), and *Scenedesmus obliquus* (11–55 wt%), which are suitable for biodiesel production.

Proteins of microalgae can be utilized for long-chain alcohols (e.g., butanol, isobutanol, isopentanol, and etc.) production through deamination of protein hydrolysates into various keto acids through metabolic engineered *Escherichia coli* (Huo et al., 2011). It was demonstrated that microalgae *Chlorella vulgaris* (51–58 wt%), *Dunaliella Bardawil* (57 wt%), *Scenedesmus Obliquus* (50–56 wt%), and *Arthrospira Maxima* (60–71 wt%) are feasible to produce up to 4.035 mg/L of long-chain alcohols (i.e., $C \geq 4$) from 22 g/L of amino acids. However, proteins are still undesirable for biofuels production due to the low concentration of fuel-convertible

amino acids in protein hydroxylates and the complexity in controlling the metabolic networks for transamination and deamination cycles. Hence, microalgal proteins are often used as nutrition sources for nutraceuticals and food additives that could enhanced the nitrogen nutrient sequestration (Bi and He, 2013). Also, the production of biomaterials and chemicals (e.g., bioplastic and biocomposites) from microalgal proteins could yield a sustainability bio-fixation of carbon cycle (Laurens et al., 2017).

2.2 Factors to consider for microalgae strain selection

Generally, the performance of bioreaction and downstream process are dependent on the microalgae strain selection during upstream cultivation. According to Fresewinkel et al. (2014), there are three main screening criteria to select the desired microalgae strains, which include: (1) biomass productivity, (2) process stability during cultivation, and (3) targeted product recovery. By using a suitable microalga strain, the effectiveness of the overall microalgae based biorefinery process could be further enhanced. The first approach for an effective microalgal cultivation is the microalgae could grow at a fast rate for high biomass density accumulation.

It is also vital to consider the mechanical and physiochemical properties of microalgae cells during the selection of a suitable process-oriented microalgae strain. By having a robust microalgae species during cultivation (especially against the shear stress and contamination induced from the cultivation system), the microalgae could be easily sustained in the medium for multiple cycles without significant interference from the surrounding environment (Rodolfi et al., 2009). This would certainly benefit the microalgae-based biorefinery as high biomass productivity could broaden valuable biochemical products production. The third indicator is the product recovery from

microalgal biomass through extraction or cell disruption. This can be enhanced by selecting microalgal species with good separation ability and required low energy input for cells disruption (Günerken et al., 2015).

On the other hand, the microalgal cultivation system can be categorized into open raceway ponds (ORP) and closed photobioreactor (PBR). The targeted microalgal cultivation parameters are varied according to the cultivation system. For example, in the ORP cultivation system, the microorganism contamination and competition are among the critical factors that influence the survival rate of microalgae cells (Cairns et al., 1972). Therefore, microalgae species strain that has high tolerance towards predators and bacteria is preferred. On the other hand, for the PBR cultivation system, the major problem encountered is the adhesion of microalgae cells on the wall of PBR (Zerriouh et al., 2017). This can be solved by choosing microalgae species with highly suspended characteristics. The controlling of the macronutrients such as nitrogen (N) and phosphorus (P) are important in sustaining the growth of microalgae cells (Barsanti and Gualtieri, 2014). The biochemical composition in microalgal biomass can be controlled via nutrients starvation approach such as controlling the N to P ratio in the culture medium (Schnurr et al., 2013). However, the nutrients starvation approach will cause distortion in cells morphology and physiology that eventually suppress the growth microalgae and resulted in low biomass productivity (Dutta Sinha et al., 2017). For example, Rasdi and Qin (2015) studied on the synergetic effects of N:P ratios on the biochemical composition for *Nannochloropsis oculata* and *Tisochrysis lutea*. It was found that the protein content was significantly affected by N:P ratios at 20:1, whereas reduction in protein synthesis accompanied by increase in lipid content was reported at the N:P ratios at 120:1 for both microalgae. Similar findings also reported in previous studies by Geider and La Roche (2002) and Lee et

al. (2013) that biochemical constituents of microalgae can be altered by strategically manipulation of N:P ratio.

As a result, the upstream bioprocess represented by the microalgal cultivation is playing a crucial role in determining the downstream end-products. It was often initiated by screening and then genetically engineered of a suitable microalgae strain for the targeted biorefinery routes. The biochemical properties of microalgal biomass are influenced by their respective cultivation conditions. Hence, by controlling the algae cultivation parameters, the distribution of the biochemical properties of microalgal biomass could be controlled in order to meet the specific demands of end-products.

2.3 Conceptual study on microalgae growth in large-scale system

In the present study, there are a few important growth parameters that need to be optimized prior to the investigation of the proposed gas-liquid mass transfer phenomenon in the designated pilot-scale BC-PBR cultivation system. The cultivation of microalgae was divided into two stages, in which the first 15 days of microalgae growth is through batch cultivation mode, and the next 15 days is semi-continuous cultivation mode. During the semi-continuous mode, 15 L of the culture is harvested every 5 days and being top-up with tap water.

Theoretically, the microalgal growth performance in most of the large-scale cultivation system is greatly influenced by both kinetic and mass transfer process (Baquerisse et al., 1999, Molina Grima et al., 1999, Sánchez Mirón et al., 1999). Hence, the microalgal growth in the designated pilot-scale BC-PBR cultivation system

is hypothesized to be a complex combination of both kinetic and mass transfer processes (Figure 2.1).

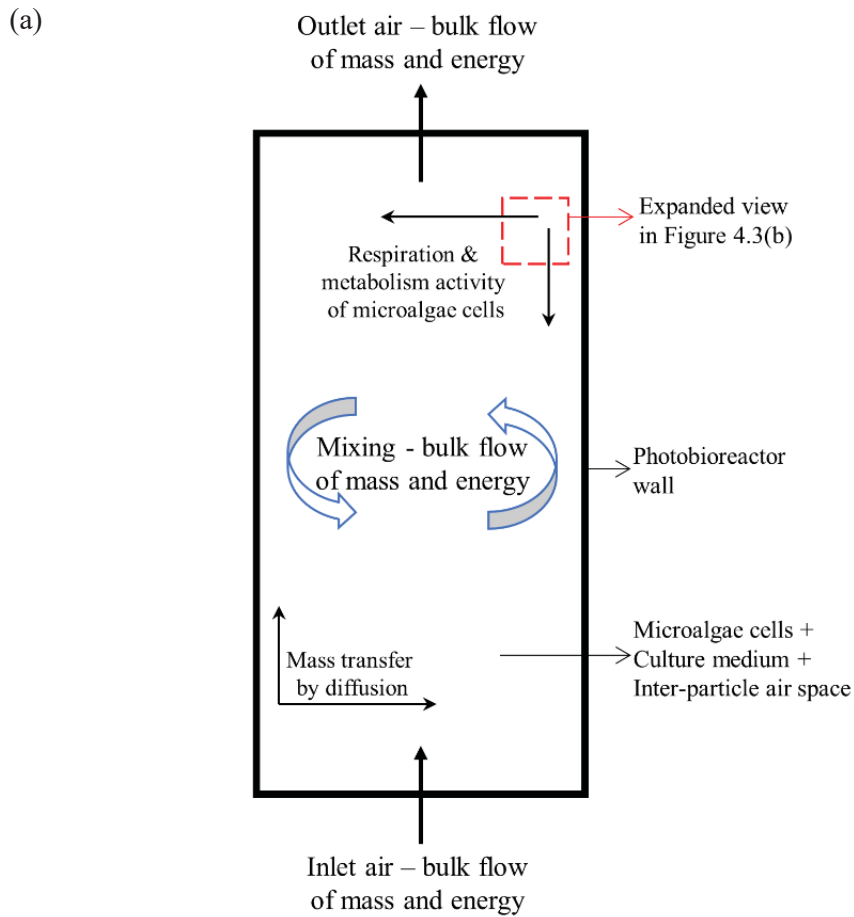


Figure 2.1 Phenomena occurring during microalgae cultivation within photobioreactors: (a) macroscale transport phenomena and (b) microscale kinetic growth for microalgae cells.

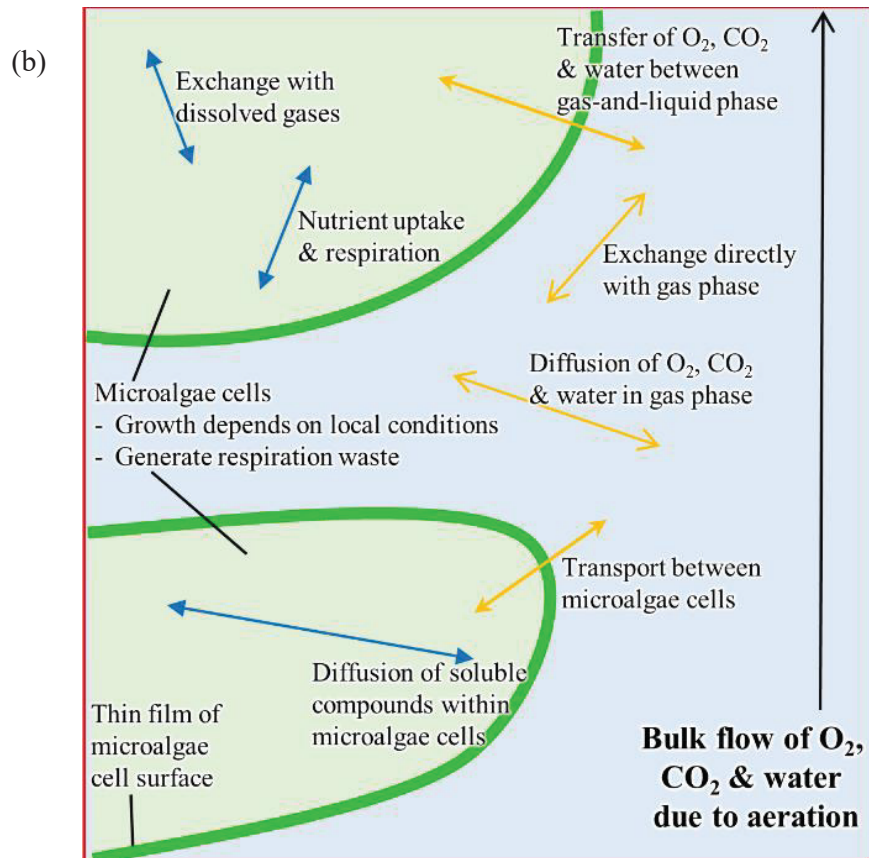


Figure 2.1 Continued

Figure 2.1(a) illustrates the mass transfer phenomenon (i.e., macroscale mass transfer by diffusion) occurring in the BC-PBR cultivation environment. Mass transfer phenomenon incorporated the mixing of bulk flow of mass and energy between microalgae cells, culture medium and inter-particle air space (void) such as gas-liquid mass transfer between microalgae cells and culture medium, and diffusion of gas solute into the culture medium. Whereas Figure 2.1(b) represents respiration and metabolism activity of microalgae cells (i.e. microscale kinetic growth activity), which included diffusion of soluble compounds, nutrients uptake and respiration by microalgae at the intracellular level. Based on the hypothesis proposed, the major macroscale and microscale phenomena identified in this particular context of study are gas-liquid mass transfer between microalgae cells and culture medium incorporated with the kinetic growth of microalgae cells.

Normally, the survivability rate of microalgae is strongly influenced by inoculum concentration, illumination intensity and cycle, and aeration rate. Hence, it is important to optimize these parameters before investigating the mass transfer influence towards microalgae growth system. The investigation on the influences of mass transfer phenomenon and the relatively hydrodynamic stress induced in pilot-scale BC-PBR cultivation system is required. These investigations are needed in order to deepen the understanding of the underlying mechanisms towards microalgae growth.

2.4 Microalgal culturing factors

Generally, the microalgal culturing parameters can be categorized according to abiotic, biotic, and operational factors, as tabulated in Table 2.1.

Table 2.1 Categories of microalgal culturing parameters.

Factors	Microalgal growth parameters
Abiotic	Light Temperature Nutrients supply (e.g., N, P, K, etc.) CO ₂ and O ₂ concentration pH Salinity
Biotics	Microorganism contamination (e.g., bacteria, viruses, fungi, etc.) Competition with other microalgae species for abiotic matters
Operational	Addition of bicarbonates Mixing and stirring degree Dilution ratio Aspect ratio (i.e. vessel width and depth) Harvest frequency

The optimal parameters for all three factors are species-specific and interdependent to each other, indicating that optimal parameters would vary widely with culture conditions. Hence, an optimum microalgae culturing system (i.e., open, closed and hybrid microalgal cultivation systems) requires a trade-off within all key parameters in order to sustain the optimum microalgae growth and productivity.

2.4.1 Illumination

Light is the major source of energy for microalgal photosynthesis reactions, which greatly affects the microalgae growth and their respective biochemical constituents productivity. The efficiency of light can be measured quantitatively (i.e., illumination intensity and photoperiod cycles) and qualitatively (i.e., spectral quality).

Among all, the illumination intensity has the most significant influence in controlling microalgae growth, where the microalgae photosynthetic rate, P is correlated with light intensity, I (i.e. photosynthetic-intensity (PI) response) (Béchet et al., 2013). Figure 2.2 illustrates a typical PI response curve (i.e., the dependency of the microalgae photosynthetic rate on light intensity), which can be categorized into three regions – light-limited, light-saturated and light-inhibited. At limited-light region, the photosynthetic rate is limited by the capture of photons emitted from low light intensities, yielding a direct proportional relationship of PI . The slope of the curve, α represents the maximum light intensity utilization efficiency, whereas the intersection between the maximum photosynthesis rate, P_{max} with α indicated the saturation threshold of light intensity, I_k . As soon as the light intensity reached I_k , the P would be at its maximal rate (P_{max}), and independent to light intensity. Under this

condition, the photosynthetic rate is limited by the reaction rate. If I reached beyond an inhibitory threshold (I_{inhib}), P will decrease due to the deactivation of photosynthetic proteins in microalgae cells. The PI response was supported by investigation done by Zhu (2015), who concluded that too high illumination intensity (i.e., exceed the light saturation point) would cause photo-inhibition, whereas the low intensity of light (i.e., below the compensation point) would limit microalgae growth rate. On the other hand, the PI response is interdependent on the depth of culture and culture density. Wahidin et al. (2013) reported that at higher cell concentration with deeper cultivation system depth, higher intensity of illumination is required to penetrate through the microalgal culture.

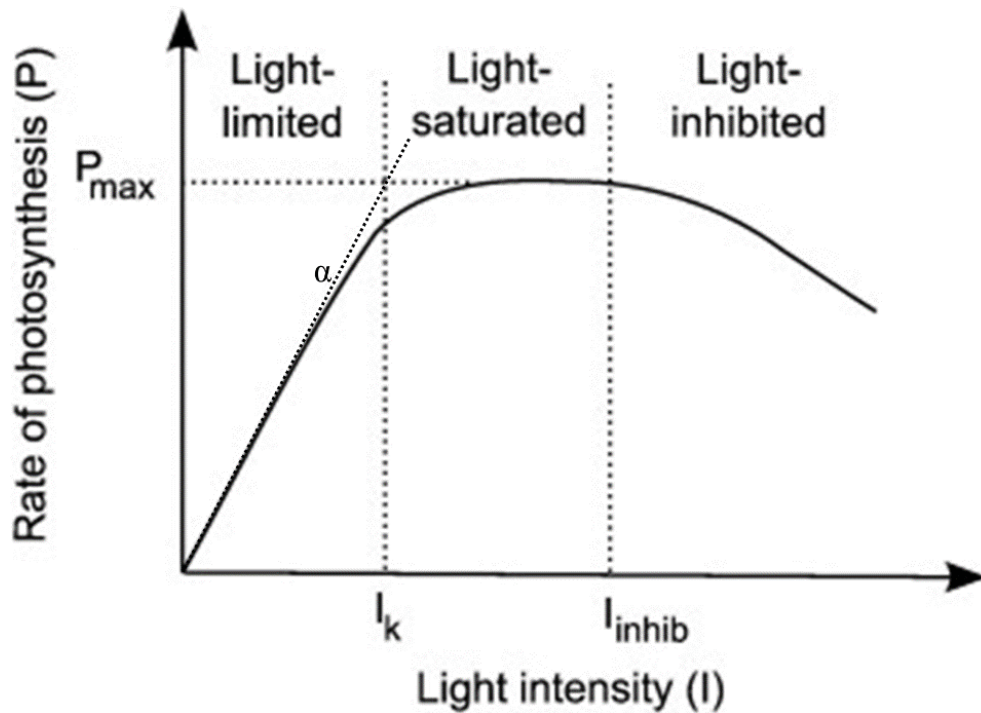


Figure 2.2 The schematic diagram of a typical photosynthetic-intensity (PI) response curve (i.e., the dependency of microalgae photosynthetic rate on light intensity). Adapted from Béchet et al. (2013).

The photoperiod is important in determining the most efficient light/dark cycles for microalgal photosynthetic conversion and then resulting in improving biomass productivity. Sarat Chandra et al. (2017) investigated the effect of photoperiod on microalga *Scenedesmus obtusus* biomass yield under various photoperiods (i.e. 12:12, 16:8 and 24:0 Light/Dark cycles) in a 3.4 L airlift photobioreactor. The results indicated that maximum biomass yield (0.836 g L^{-1}) can be achieved under continuous light condition. However, the photoperiod experienced an inversely proportional relationship with light intensity. This finding was supported by Yan et al. (2016) who investigated on lighting control strategy for optimized microalgal growth. They reported that highest microalgal biomass accumulation (483 and 390 mg L^{-1}) with different lighting control strategy respectively (i.e., low light intensity ($300 \mu\text{mol m}^{-2} \text{ s}^{-1}$) with long photoperiod (16:8 light/dark cycle), and vice versa ($900 \mu\text{mol m}^{-2} \text{ s}^{-1}$; 12:12 light/dark cycle)).

The light spectrum that suitable for microalgae photosynthesis is defined as Photosynthetically Active Radiation (PAR), which is ranging from 400–700 nm (i.e., closely to visible spectrum region). The natural light is sourced from solar radiation that consists of 43% of the visible spectrum (Ringsmuth et al., 2016). Hence, a fluorescent light that emitted visible light is one of the suitable artificial lighting sources for microalgae photosynthesis process. Meanwhile, the optimization of spectral light quality has been reported to be effective in enhancing the microalgal growth and their respective desired product of interest for every microalgal strain individually. From Seo et al. (2014) study, it was found that microalga *Chlorella* sp. reached maximum microalgal biomass accumulation (1.7 g/L) and lipid productivity (30 wt%) under red and blue light spectrum respectively, in a 350 mL two-layer cultivation reactor. In addition to that, Kim et al. (2017) investigated the influence of

selectively transmitting spectral light regions (i.e. red, blue, and red+blue) to cultivate microalgae *Tetraselmis* sp. in a 400 mL bubble column photobioreactors. Their findings indicated that the microalgal biomass and fatty acid productivities of microalgae *Tetraselmis* sp. were increased by 7–53% and 9–61% respectively, under red light illumination.

2.4.2 Culturing temperature

The microalgae culture should maintain at their localized habitat temperature, which can be classified according to polar organisms (<10 °C), temperate (10-25 °C), and tropical (>20 °C). Most of the laboratory cultures can tolerate a temperature range from 16 to 27 °C, but vary depending on the microalgae strains, species, and nutrient medium of the culture used. However, the growth performances of microalgae are extremely sensitive to low or high-temperature environment, especially during outdoor cultivation. For instances, the growth rate would significantly reduce by culturing microalgae at a temperature below 16 °C; meanwhile, cultivation environment with the temperature higher than 35 °C are lethal for microalgae growth (Ras et al., 2013).

From microalgae biochemical composition analysis, it was found that lipid content was strongly influenced by culture temperature. Microalgae *Nannochloropsis oculata* and *Chlorella vulgaris* illustrated the temperature influence on lipid accumulation. It was found that lipid content of *N. oculata* increases two-fold, from 7.90 to 14.92 % with increasing temperature from 20 to 25 °C, but showing depreciation in *C. vulgaris* lipid content from 14.71 to 5.90 % as the temperature was further increased from 25 to 30 °C (Converti et al., 2009).

2.4.3 Nutrients

Microalgae culture medium require macronutrients (i.e. nitrogen (N), phosphorus (P) and potassium (K)) and micronutrients such as trace metals (e.g., iron, zinc, copper, cobalt, magnesium, etc.) and vitamins (e.g., vitamin B₁, B₇, and B₁₂) to sustain the growth of microalgae population (Cheng and He, 2014). The nutritional deficiency would cause distortion in cells morphology and physiology that suppressed the growth and developments of microalgae, and caused a decline in biomass productivity.

According to Barsanti and Gualtieri (2014), N and P are the two major elements that can immediately retard the growth of photosynthetic microalgae cells. N is required for biosynthesis of internal structures (including nucleus acids, proteins and photosynthetic pigments) of microalgae cells, whereas P is the main elements required for the cellular metabolic process. N-limitation would influence the supply of amino acids, causing a reduction in photosynthetic rates and lead to a decline in respiratory rates. Besides that, P-limitation could reduce the protein synthesis rate in photosynthetic cells, causing inhibition of protein synthesis in microalgae cells, and thus affect the metabolism of the cells. Additionally, P also plays a vital role in the Calvin cycle in order to synthesise and regenerating substrates for microalgae growth.

Apart from that, the biochemical composition in microalgal biomass can be control via selected nutrients limitation approach such as controlling the N to P ratio in the culture medium. For example, Rasdi and Qin (2015) studied on the synergetic effects of N:P ratios on the biochemical composition for two different microalgae species (i.e. *Nannochloropsis oculata* and *Tisochrysis lutea*). They reported that protein content was significantly affected by N:P ratios at 20:1, whereas a reduction in

protein synthesis accompanied by increase in lipid content was discovered at the N:P ratios at 120:1 for both microalgae. Similar findings also reported in previous studies by Geider and La Roche (2002) and Lee et al. (2013), indicated that biochemical constituents of microalgae can be altered by strategically manipulated N:P ratio.

For micronutrients, iron (Fe) is the key element for photosynthetic electron transport chain that would affect the photosynthesis process. Sosik and Olson (2002) reported that photosynthesis efficiency could be affected by Fe-deficient condition, which is required to regulate the physiology of the microalgal cell that controls biomass productivity. This finding was further supported by Park et al. (2013), who elucidate the photosynthetic characteristics of microalgae as an index of Fe-limitation.

2.4.4 pH and salinity

Generally, in most of the laboratory, microalgae are cultivated at pH 7, with some exceptional species can grow in more acid or alkaline environments. However, the pH values of the culture medium will fluctuate during the entire cultivation period, due to the concentration of CO₂ remaining in the culture medium.

Several works of literature had reported the influences of pH and salinity for microalgae growth on their respective biomass accumulation and biochemical composition. Goldman et al. (1982a) studied four different types of microalgae (*Scenedesmus obliquus* and *Chlorella vulgaris* as freshwater species, whereas *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* as marine species) and concluded that pH tolerance limitation is influenced by both metabolic activity and environmental factors, especially in large-scale outdoor cultivation. In a subsequent study by Goldman et al. (1982b), it was claimed that pH would influence the biomass