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

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

by

KHOO CHOON GEK

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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

Kee, Rong-Er, and Sin Mun), who provided a much-needed form of escape from my studies, also deserved thanks for helping me keep things in perspective. Thank you for having me over and for the moral support in one way or another, making this an experience that I will cherish forever. My deep appreciation also goes out to Yee Jie and his family for their great hospitality and warmth they extended to me during my time in Penang and for always making me feel so welcome.

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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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
$d_{b,\,max}$	Maximum bubble diameter	mm
d_0	Diameter of gas sparger hole	mm
D	Diffusivity	$m^2\ s^{-1}$
D_{O_2}	Diffusivity of oxygen, O_2 at 298 K	$m^2\ s^{-1}$
D_{CO_2}	Diffusivity of carbon dioxide, CO_2 at 298 K	$m^2\ s^{-1}$
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
$k_G a_L$	Volumetric gas phase mass transfer coefficient	s^{-1}
$K_L a_L$	Overall volumetric gas-liquid mass transfer	s^{-1}
$k_L a_L$	Volumetric liquid phase mass transfer coefficient	s^{-1}
K	Solubility products	
K_H	Henry;s Law constant	Dimensionless
$K^{\circ}_{H_2CO_3}$	Solubility constant of carbonic acid	Dimensionless
$K^{\circ}_{HCO_3^-}$	Solubility constant of HCO_3^-	Dimensionless
K°_{w}	Solubility constant of water	Dimensionless
LPM	Unit for compressed air aeration flow rate	L min^{-1}
N	Microalgal biomass density	g/L
OD_{540}	Optical density at wavelength of 540 nm	Dimensionless
P	Microalgal biomass productivity	mg L^{-1} 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 CO_2 L^{-1} 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	^oC
T_b	Burnout temperature	^oC

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)	L L^{-1} min^{-1}
$x^*_{CO_2}$	CO_2 equilibrium mole fraction in solution	mol
$y^*_{CO_2}$	CO_2 equilibrium mole fraction in the gas phase	mol
$y^{*\prime}_{CO_2}$	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 (CO_2) 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 ^{o}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 µmol m^{-2} s^{-1}, dan kadar pengudaraan 0.16 vvm. Pekali pemindahan jisim gas-cecair, $k_La_L(CO_2)$ 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 ^oC 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 pengulturan BC-PBR secara separa berterusan pada skala perintis. Seterusnya, ia mempengaruhi kualiti biojism 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 CO_2 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 ^{o}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 µmol m^{-2} 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, $k_L a_L(CO_2)$ 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 ^oC 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 thrid 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 (CO_2), 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 speciese 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 CO_2 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 microlagal 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 supressed 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 haboring 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, CO_2 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 placed 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. Additonally, 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 CO_2 fixation rate, which would not hinder the mass transfer results since the ability of cells to utilize maximum amount of CO_2 that dissolved in microalgae culture (in the case for atmospheric CO_2 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 ^{o}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 tricornutum* (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%), *Dunalliela 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 (Zeriouh et al., 2017). This can be solved by choosing amicrolgae 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 oculate* 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 hypotheses to be a complex combination of both kinetic and mass transfer processes (Figure 2.1).

(a)

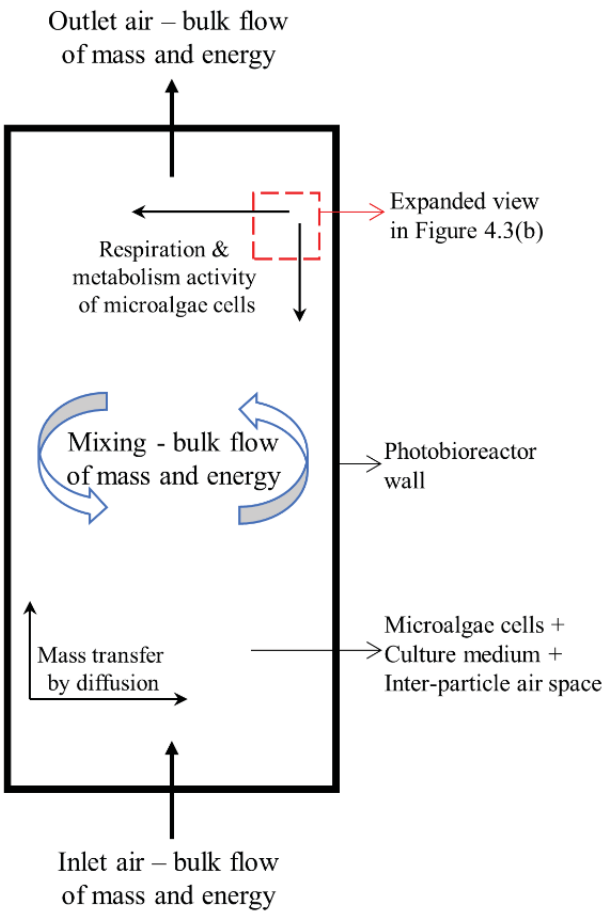

Figure 2.1 Phenomena occurring during microalgae cultivation within photobioreactors: (a) macroscale transport phenomena and (b) microscale kinetic growth for microalgae cells.

(b)

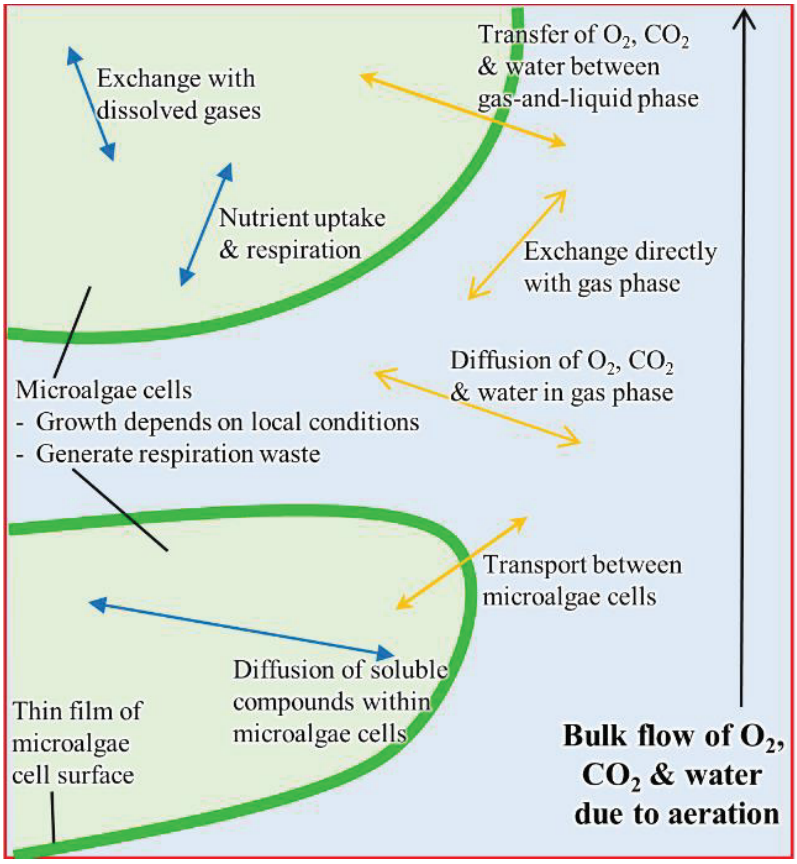

Figure 2.1 Continued

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 categories 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.

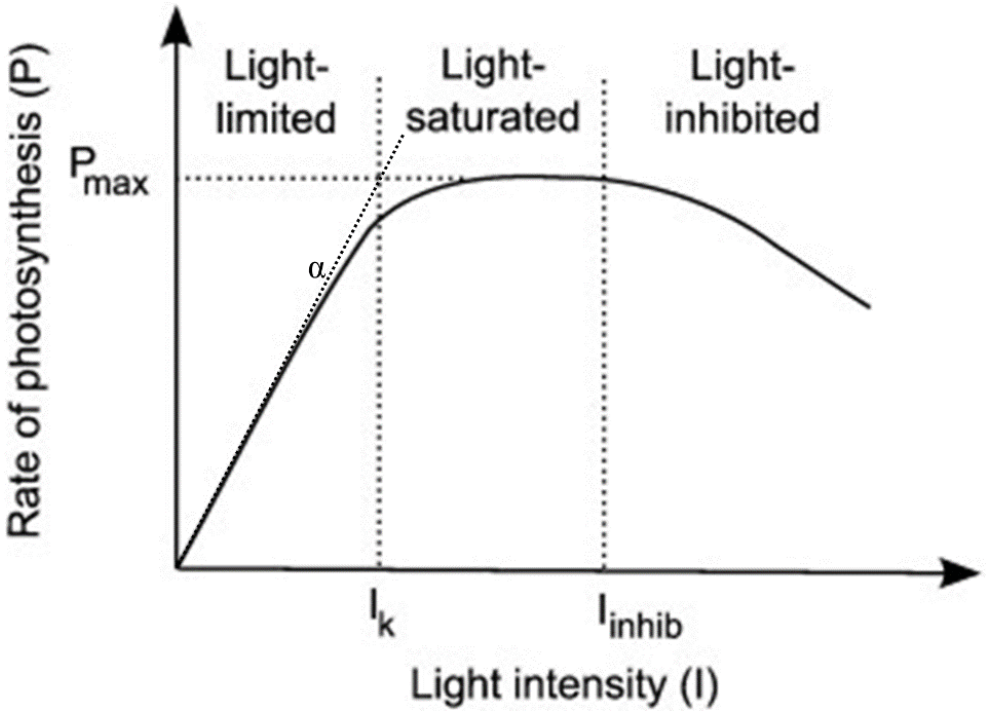

Figure 2.2 The schematic diagram of a typical photosynthetic-intensity (PI) response curve (i.e., the dependency of microalgae photosynthetic rate on light intensity). Adapted from Béchet et al. (2013).

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 μmol m^{-2} s^{-1}) with long photoperiod (16:8 light/dark cycle), and vice versa (900 μmol m^{-2} 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 microalga *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 ^{o}C), temperate (10-25 ^{o}C), and tropical (>20 ^{o}C). Most of the laboratory cultures can a tolerate a temperature range from 16 to 27 ^{o}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 ^{o}C; meanwhile, cultivation environment with the temperature higher than 35 ^{o}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 ^{o}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 ^{o}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 B_1, B_7, and B_{12}) 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 oculate* 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 CO_2 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