



***EFFECTS OF DIFFERENT LIGHT SPECTRUMS ON GROWTH AND
PROXIMATE BIOCHEMICAL COMPOSITION OF *Chaetoceros calcitrans*
AND MARINE *Chlorella* sp.***

GREGORY TAN GUAN YUAN

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By

GREGORY TAN GUAN YUAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

December 2019

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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December 2019

Chairman : Sanjoy Banerjee, PhD
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The growth and biochemical composition of microalgae is influenced by light, CO₂, nutrients, temperature, pH and mixing. The spectral quality of light source plays an important role in the growth of microalgae and their biochemical composition. Light emitting diodes (LEDs) are an alternative for replacing sunlight and less energy efficient conventional lights as the spectral wavelengths of LEDs can be customized according to species requirements and they consume much less energy.

The aim of this study is to examine the growth, proximate biochemical composition and fatty acid profile of *Chaetoceros calcitrans* and marine *Chlorella* sp. In the first phase, microalgae were cultivated in small scale photobioreactors (1 L) and exposed to five different spectrums from LEDs – blue, white, green, yellow, red and fluorescent lamp (FL) as the control. In *C. calcitrans*, yellow light demonstrated the highest ($p < 0.05$) specific growth rate. Protein content was found to be the highest in the FL-control treatment followed by blue and red light, and lowest in yellow light. Lipid content in *C. calcitrans* was also found to be amongst the highest in yellow along with blue and green light but lowest in white light. Green light treatment had the highest carbohydrate content over the other treatments whereas the lowest was found in white light. The fatty acid content was also found to be influenced by light spectrums. *Chaetoceros calcitrans* produced highest amounts of ω 3 essential fatty acids under red light whereas it was green light for poly-unsaturated fatty acids (PUFAs). Based on the high specific growth rate and lipid production from the first phase, yellow light was selected for the second phase of the experiment which was conducted in flat panel photobioreactors (FP-PBR; 60 L). In phase 2, no significant difference was found in cell density and biomass upon harvest in both yellow and FL-control. However, yellow light demonstrated a higher protein content over FL-control, but showed no

significant difference in lipid and carbohydrate. Yellow light also produced the highest PUFAs and ω 3 fatty acids.

As seen for *C. calcitrans*, yellow light also demonstrated a higher specific growth rate in marine *Chlorella* sp. in phase 1. The highest protein content in *Chlorella* sp. was found in red light while the lowest was in FL-control treatment. However, FL-control showed the highest lipid content. Lowest carbohydrate content was found in red light. In *Chlorella* sp., red light produced higher amounts of ω 3 and PUFAs and green light promoted the synthesis of ω 6 fatty acids. Based on the high specific growth rate of *Chlorella* sp. from the phase 1, yellow light was selected for phase 2 of the experiment which was conducted in flat panel photobioreactors (FP-PBR; 60 L). In phase 2, no significant differences were found in cell density and biomass upon harvesting in both yellow and FL-control. However, yellow light only demonstrated a higher protein content over FL-control, and was significantly lower in lipid and carbohydrate content. Yellow light also produced the highest PUFAs and ω 3 fatty acids in *Chlorella* sp.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN SPEKTRUM CAHAYA BERBEZA TERHADAP PERTUMBUHAN
DAN KOMPOSISI BIOKIMIA PROXIMASI *Chaetoceros calcitrans* DAN
Chlorella sp. MARIN**

Oleh

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Pertumbuhan dan komposisi biokimia mikroalga dipengaruhi oleh cahaya, karbon dioksida, nutrien, suhu, pH dan peredaran. Kualiti spektrum sumber cahaya memainkan peranan penting dalam pertumbuhan mikroalga dan komposisi biokimia mereka. Pancaran cahaya diod (LED) adalah sumber cahaya alternatif untuk menggantikan cahaya matahari dan lampu konvensional yang kurang cekap dan panjang gelombang spektrum LED boleh diubahsuaikan mengikut keperluan spesies dan mereka menggunakan tenaga yang kurang.

Tujuan kajian ini adalah untuk mengkaji pertumbuhan, komposisi biokimia proksima dan profil asid lemak *Chaetoceros calcitrans* dan *Chlorella* sp. marin. Pada fasa pertama, mikrolalga dikultur dalam fotobioreaktor kecil (1 L) dan terdedah kepada lima spektrum LED yang berlainan - biru, putih, hijau, kuning, merah dan lampu pendarfluor sebagai kawalan. Bagi *Chaetoceros calcitrans*, cahaya kuning menunjukkan kadar pertumbuhan spesifik ($p < 0.05$) yang tertinggi. Kandungan protein *C. calcitrans* didapati paling tinggi dalam rawatan kawalan diikuti oleh cahaya biru dan merah, dan paling rendah dalam cahaya kuning. Kandungan lipid dalam *C. calcitrans* juga didapati tertinggi dalam cahaya kuning bersama dengan biru dan hijau tetapi paling rendah dalam cahaya putih. Rawatan cahaya hijau mempunyai kandungan karbohidrat tertinggi berbandingkan rawatan lain manakala yang paling rendah didapati dalam cahaya putih. Kandungan asid lemak juga didapati dipengaruhi oleh spektrum cahaya. *Chaetoceros calcitrans* menghasilkan $\omega 3$ asid lemak penting yang tertinggi di bawah cahaya merah manakala cahaya hijau untuk asid lemak poli tidak jenuh. Berdasarkan kadar pertumbuhan spesifik dan pengeluaran lipid yang tinggi dari fasa pertama, cahaya kuning dipilih untuk eksperimen fasa kedua yang dijalankan di dalam fotobioreaktor panel rata (60 L). Dalam fasa 2, tidak mempunyai perbezaan yang ketara dalam ketumpatan sel dan biomas semasa penuaian di antara

kuning dan kawalan. Walau bagaimanapun, cahaya kuning menunjukkan kandungan protein yang lebih tinggi berbanding dengan kawalan, tetapi tidak menunjukkan perbezaan ketara dalam lipid dan karbohidrat. Cahaya kuning juga menghasilkan asid lemak poli tidak jenuh dan asid lemak $\omega 3$ yang tertinggi.

Seperti yang dilihat untuk *C. calcitrans*, cahaya kuning juga menunjukkan kadar pertumbuhan spesifik yang lebih tinggi di dalam *Chlorella* sp. marin dalam fasa 1. Kandungan protein tertinggi dalam *Chlorella* sp. didapati dalam cahaya merah manakala yang paling rendah adalah kawalan. Walau bagaimanapun, kawalan menunjukkan kandungan lipid tertinggi. Kandungan karbohidrat yang paling rendah didapati dalam cahaya merah. Dalam *Chlorella* sp., cahaya merah menghasilkan $\omega 3$ dan asid lemak poli tidak jenuh yang lebih tinggi dan cahaya hijau menggalakkan sintesis asid lemak $\omega 6$. Berdasarkan kadar pertumbuhan spesifik *Chlorella* sp. yang tinggi dari fasa 1, cahaya kuning dipilih untuk dijalankan dalam eksperimen fasa 2 menggunakan fotobioreaktor panel rata. Dalam fasa 2, tidak terdapat perbezaan yang ketara dalam ketumpatan sel dan biomas semasa penuaian di antara kuning dan kawalan. Walau bagaimanapun, cahaya kuning hanya menunjukkan kandungan protein yang lebih tinggi berbanding dengan kawalan, dan lebih rendah ketara dalam kandungan lipid dan karbohidrat. Cahaya kuning juga menghasilkan asid lemak poli tidak jenuh dan asid lemak $\omega 3$ yang tertinggi dalam *Chlorella* sp.

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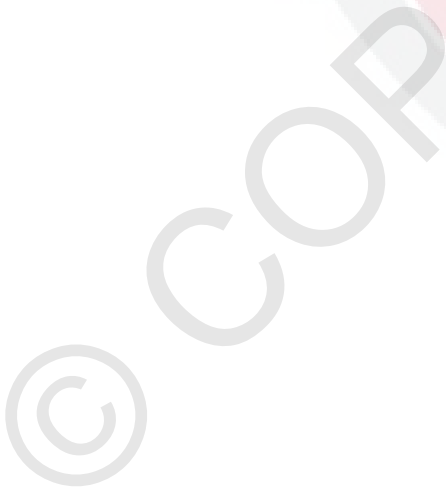
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LIST OF ABBREVIATIONS

ALA	α -linolenic acid
ALR	Airlift reactor
Ast	Astaxanthin
BBM	Bold Basal medium
BG-11	Blue green medium
BSA	Bovine serum albumin
Car	Carotenoids
Chl	Chlorophyll
cm-LED	colour-mixed-LED
d.c.	Duty cycles
DHA	Docosahexaenoic acid
DO	Dissolved oxygen
DPA	Docosapentaenoic
DW	Dry weight
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FID	Flame ionization detector
FL	Fluorescent lamps
FP-PBR	Flat panel photobioreactor
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
LC-PUFA	Long-chain polyunsaturated fatty acids
LED	Light emitting diode

LHP	Light harvesting protein-pigments
Lut	Lutein
Lyc	Lycopene
MUFA	Monounsaturated fatty acids
PAR	Photosynthetic active radiation
PBR	Photobioreactor
PCE	Power conversion efficiency
PCR	Polymerase chain reaction
pc-LED	Phosphor-converted-LED
PEP	Phosphoenolpyruvate
pH	Potential of hydrogen
Phy	Phycocyanin
PPFD	Photosynthetic photon flux density
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SE	Standard error
SFA	Saturated fatty acids
SGR	Specific growth rate
OD	Optical density
TCA	Trichloroacetic acid
UV	Ultra-violet
Zea	Zeaxanthin
β-car	β-carotene

LIST OF NOTATIONS

μmol	Micromole
m^2	Square meter
m	Meter
cm	Centimeter
μm	Micrometer
nm	Nanometer
mm^2	Square millimeter
cm^3	Cubic centimeter
s	Seconds
m^2h^{-1}	Square meter per hour
kWh^{-1}	Kilo watts per hour
%	Percent
g	Grams
kg	Kilogram
pg	Picogram
mg	Milligram
L	Liter
μL	Microliter
mL	Milliliter
C_t	Concentration at time
C_0	Initial concentration
e^{mt}	Exponential with the function of specific growth and time
lux	One lumen per square meter
$^{\circ}\text{C}$	Celsius
ppt	Parts per thousand
λ_e	Emission peak

λ	Wavelength
W	Watt
InGaN	Indium gallium nitride
AlGaInP	Aluminium gallium indium phosphide
FWHM	Full width at half maximum
€	Euro currency
$\mu\text{molm}^{-2}\text{s}^{-1}$	Micromole per square meter per second
$\mu\text{mol-ph s}^{-1} \text{W}^{-1}$	Micromole of photon per second per watt
h	Hour
Wm^{-3}	Watts per cubic meter
N	Normality
min	Minutes
rpm	Revolution per minute
X_2	Final cell density
X_1	Initial cell density
$t_2 - t_1$,	Time/duration of exposure
R^2	Proportion of the variance for a dependent variable in a regression model
$\mu\text{g mL}^{-1}$	Microgram per milliliter
m	Slope standard curve
A	Absorbance
c	y-intercept
p	Significant level
n	Number of replicates
ω_3	Omega 3
ω_6	Omega 6
kHz	Kilohertz

CHAPTER 1

INTRODUCTION

1.1 Background of study

Microalgae are fast-growing microorganisms (Gordon and Polle, 2007) found in a wide range of habitats including marine, freshwater, and brackish water (Abbott et al., 1992; Wehr, 2002). They are characterized by their high productivity per unit area in comparison with higher photosynthetic plants (Carvalho et al., 2011). This notable photosynthetic efficiency is dominated by the advantages of their reduced internal physiological competitiveness, instant reproduction cycles, narrow nutrient requirements and adaptation to a wide range of radial intensities, temporal and spectral distributions (Gordon and Polle, 2007). Microalgae are widely used as a food source for fish (Reitan et al., 1994), crustacean (Preston et al., 1992), and bivalve (Frankish et al. 1991) larvae due to their high nutritional value in proximate composition as well as fatty acids. Essential fatty acids plays an important role in the initial growth of aquaculture larvae and juvenile development (Renaud et al., 1999).

Microalgae cultivation can be carried out in open ponds or closed-system photobioreactors (Singh and Sharma, 2012). Essential factors such as light, pH, CO₂, and the medium's nutrient content have to be finely attuned (Koc et al., 2013) for the optimum growth of microalgae. However, light would be the most imperative factor as it is generated by electricity in closed-photobioreactor culture systems hence affecting the production cost of biomass (Koc et al., 2013). Visible light encompasses different spectral wavelengths and is found to influence the physiological changes in cell size, reproduction and biochemical composition such as protein, polysaccharides, lipids, fatty acids, pigments and other derived compounds (Rivkin, 1989; Koc et al., 2013). For instance, biomass of *Spirulina platensis* was found to be similar when cultivated using white LED, flourescent and natural light with different spectral properties (Chen and Lee, 2012). These finding prove that alternative light sources can be used for the cultivation of microalgae. In general, many species of microalgae have higher carbon fixation rates in their cells (Rivkin, 1989), increasing their biomass when exposed to blue light. On the contrary, red light promotes cell division (Koc et al., 2013). However, responses are still species specific under different circumstances.

Photon flux density is the amount of photons in μmol striking a surface of 1 m^2 in 1s . The significance of providing an optimum photon flux density in microalgae cultures is because dense cultures are often challenging to maintain as they have the tendency to reflect, refract, and absorb light, thus impeding it from reaching the entire depth of the photobioreactor (Koc et al., 2013). If high light intensities are engaged to counter mutual shading in dense cultures, installation of a cooling system might be necessary as the light source could accelerate high temperatures which may inhibit growth and even kill cells (Park and Lee, 2000). This would eventually add on to biomass production costs. Excessive light may have negative effects on microalgae (or plants)

where photons cannot be further absorbed by photoreceptors, a phenomenon known as photo-saturation (Koc et al., 2013). This could subsequently lead to photo-inhibition and photo-oxidation, resulting in photosynthesis retardation and even damaging cells; although the photons exist within the photosynthetic active radiation (PAR) range which falls between 380 and 750 nm (Koc et al., 2013). Contrarily, photosynthesis will also be deterred if light intensities are too low as a consequence of shading; henceforth reducing biomass production (Koc et al., 2013).

The PAR is an important factor in photosynthesis as microalgae utilizes the spectral wavelengths within its range (Koc et al., 2013). The PAR affects microalgal production and has to be given due considerations in the design of lighting systems for photobioreactors (Koc et al., 2013). LED's application in photobioreactors (PBRs) demonstrated a great advancement over existing indoor agricultural lighting (Yeh and Chung, 2009). The incorporation of LED lights into PBR designs was initiated in the early 1990s (Lee and Palsson, 1994). LEDs are amongst the preeminent alternatives available in the current lighting technology to be adopted by PBRs (Fu et al., 2012). Higher luminous efficiency, lower energy consumption, and longer life span are some of the major advantages LEDs can offer over other light sources (Chen and Lee, 2012). LEDs are light and small enough to fit into virtually any culture system, such as plant incubators and photobioreactors (Chen and Lee, 2012), and they permit the alteration of spectral quality as well as quantity in optimizing illumination. LEDs have narrow spectral bands between 20-30 nm (Chen et al., 2010), producing desirable wavelengths at higher energy conversion efficiency (Yam and Hassan, 2005). LEDs can also minimize abiotic stresses rising from excessive illumination upon cells and they are easily obtainable. Their small structure, low energy consumption, and ease of installation make LEDs more environmental-user friendly than other light sources (Koc et al., 2013). In addition, LEDs do not produce as much heat as other types of lights, thus preventing overheating of the culture (Koç et al., 2009). Therefore, LED lighting technology is a practicable option for microalgal cultivation, especially in speeding up the production rate of biomass and specific biochemical compounds (Schulze et al., 2014).

Photobioreactors (PBRs) precedes the benefits of open systems as it assures higher culture purity, reduces the use of water, CO₂ loss and better control over environmental conditions such as pH, temperature, light and other factors (Singh and Sharma, 2012). Besides, they also permit higher biomass quantity and fine biopharmaceutical production (Singh and Sharma, 2012). Commercial microalgal cultivations are commonly carried out in open-pond systems (Chen et al., 2010). However, the performance of open-pond cultures is usually less satisfactory. This is because culture conditions including UV-radiation exposure, biological contamination, natural light intensities and distributions are difficult to control (Chen et al., 2008). These limitations are often observed throughout variations in diurnal cycles, weathers, seasons and solar spectrums (Chen et al., 2010). The maximum average productivity for outdoor open algal ponds is 1 g DW m⁻²h⁻¹, while in closed algal photobioreactors the productivity increases to 2-3 g DW m⁻²h⁻¹ (Weisz, 2004). To overcome constraints, LEDs are being integrated into closed photobioreactor systems to ensure continuous, sufficient and stable light supply (Chen et al., 2010). Most of laboratory-scale

photobioreactors are illuminated using fluorescent lamps, which entails a higher power consumption and operating cost (Chen et al., 2010). Hence, by replacing fluorescent lamps with multi-LED light, electricity consumption may be reduced up to 50 % from 40.32 to 20.16 kWh⁻¹ (Chen et al., 2010).

1.2 Problem Statement

Microalgae have recently received much attention as a new biomass source for protein, lipid, fatty acids, amino acids, vitamins and pigments which can be used as high value products. This makes microalgae a potentially rich source of chemical products with applications in food, animal feed, nutritional, cosmetic and pharmaceutical industries. High quality biomasses at cheaper costs are necessary to make products viable for commercial applications. One of the important factors in achieving high density productive microalgal culture is light. The growth of microalgae and the nutritional composition of microalgal biomass are known to be greatly dependent on the light source. Sunlight is a cheap and natural source of light normally utilized for microalgae cultivation. However, limited availability due to diurnal cycles and seasonal variations impedes the viability of commercial production. To circumvent these issues, artificial lights are commonly integrated into microalgae cultures. This would ensure uninterrupted production since continuous and controlled illumination will increase microalgal productivity. Artificial lights have limitations as well due to their spectral properties. Therefore, appropriate light sources having specific spectrum should be identified for the illumination of cultures in order to achieve optimum growth. A number of studies have been conducted on light regimes (Carvalho et al., 2011), nutrient manipulation (Mandalam and Palsson, 1998; Ramos et al., 2011), salinity manipulation (Fazeli et al., 2006) and cellular engineering (Beckmann et al., 2009) in optimizing microalgal growth, lipid accumulation (Bartley et al., 2013) and pigmentation (Ramos et al., 2011). However, few studies have been conducted on the effects of different light quality on microalgal growth and proximate composition (Abiusi et al., 2014).

Open pond cultures using natural light tend to have higher risks of failure associated with contamination, less- or uncontrollable culture conditions like direct UV irradiation exposure, low light intensity, uneven light distribution (Chen et al. 2008) and low cell density (Chen et al., 2010); therefore it is crucial to look into alternative culture systems to minimize the risk factors. Closed photobioreactor culture systems may be the solution to the problems as mentioned above. However, types of lights integrated in the PBRs and their spectral quality on how it affects the physiological properties of cells is yet to be investigated.

1.3 Significance of Study

Microalgae are significant commercially as they are cultured for food, valuable compounds, bio-remediation, pharmaceuticals, aquaculture and biofuel purpose (Lorenz and Cysewski, 2000; Brown, 2002; Sharma et al., 2013; Velichkova et al., 2014). The purpose of this study is to examine the growth rates and proximate composition of two commercially significant microalgae species *Chaetoceros calcitrans* and marine *Chlorella* sp. exposed to different light spectrums. Thus, a flat panel photobioreactor culture system (FP-PBR; Patent pending UI2014700185) with light sources from LEDs and fluorescent lamps (as control) composed of different spectral qualities, was adapted to maximize the biomass productivity (Fu et al., 2012) and lipid composition (Chen et al., 2010) of microalgae. Since spectral ranges and distribution of LEDs can be customized, it will provide significant results of the physiological changes induced by their light qualities and to compare the results with other light treatments (e.g. fluorescent lamps). The responses of microalgae to light qualities are species dependent (Gorai et al., 2014; Hultberg et al., 2014; Schulze et al., 2014); thus, specific wavelengths of energy-saving LEDs can be chosen selectively (Blanken et al., 2013), while increasing or maintaining product yields in expressions of the biomass itself or biomass-derived compounds. The intention behind spectral selection is to eliminate energy wastage used to irradiate wavelengths which are photosynthetically less efficient (Abiusi et al., 2014). This study would benefit commercial farms in microalgal production as the understanding of light quality effect on the growth and biochemical composition of microalgal species may help increase production in biomass and its derivatives more efficiently.

1.4 Objectives

This study was undertaken specifically with the following objectives:

1. To determine the growth of *Chaetoceros calcitrans* and marine *Chlorella* sp. cultivated under different light spectrums in photobioreactors.
2. To analyse the proximate composition and fatty acid profiles of the two microalgae species cultivated under different light spectrums in photobioreactors.

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